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CLOT RETRACTION AS A MEASURE OF PLATELET FUNCTION

I. Effects of Certain Experimental Conditions on Platelets in vitro

ROBERT C. HARTMANN and C. LOCKARD CONLEY

Department of Medicine, the Johns Hopkins University School of Medicine and Hospital

Received for publication September 28, 1953

The blood platelets are required for the maintenance of normal hemostasis. A number of functions have been ascribed to platelets including acceleration of coagulation, retraction of blood clots, maintenance of the integrity of capillary walls and the liberation of a vasoconstrictor substance. Of these functions only clot retraction can be brought about solely by platelets. Therefore, a quantitative measure of clot retraction can be used as a specific test of platelet function. There is good evidence that *intact* platelets are required for normal hemostasis. It is noteworthy that *intact* platelets are also necessary for the retraction of clots*. The subject of clot retraction has recently been reviewed in detail by Budtz-Olsen (2).

The routine clinical method for estimating clot retraction is too crude to serve as a satisfactory measure of platelet function. The variable red cell mass, unpredictable adherence of the clot to glass surfaces and the variable rate of thrombin formation influence the rate and degree of retraction of the blood clot. In the present study a method has been employed which eliminates these variables and provides a reproducible, semi-quantitative measure of platelet function. This technique has been employed to study the effect upon the platelets of a variety of experimental conditions.

METHODS

Blood was collected with silicone-treated apparatus from normal human subjects. Platelet-rich plasma was prepared by slow centrifugation of the blood at low temperature (3). Platelet-free plasma was similarly prepared by centrifugation at about 12,500 RPM in a refrigerated Servall angle centrifuge. In most instances acid-citrate-dextrose (ACD) solution† was added to the separated plasma in the proportion of one part to five parts

Presented in abbreviated form at the 64th Annual Meeting, The American Physiological Society, April 18, 1952.

This work was carried out under Contract AT (30-1) 1208 between the Atomic Energy Commission and the Johns Hopkins University, and was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health.

* Fonio (1) has reported that the hyalomere of platelets can produce clot retraction when separated from the granulomere by suprasonic vibrations. However, under ordinary conditions disruption of platelets leads to loss of their ability to produce retraction.

† ACD solution consists of sodium citrate 50 grams, citric acid 17.85 grams, dextrose 75 grams and distilled water to a total volume of 3000 ml.

of plasma. In some instances ACD solution was added to whole blood prior to centrifugation in the proportion of one part of the anticoagulant to four parts of blood. Platelet counts of the platelet-rich plasma were determined in duplicate by a direct method employing Rees-Ecker diluent or by the phase microscopy technique of Brecher and Cronkite (4). Plasma which had been centrifuged at high speed was examined undiluted by direct microscopy as previously described (5). The platelet concentration in this plasma was in most instances less than 100 per cmm. Plasma having any platelet concentration desired was obtained by appropriate mixing of platelet-rich and platelet-free plasma. Plasma was permitted contact only with surfaces treated with silicone except in those experiments in which the effects of glass surfaces were specifically studied. No attempt was made to maintain sterility of the plasma, except in one experiment as indicated.

Clot retraction tests were performed in silicone-treated tubes. In experiments in which plasma was stored in glass tubes, plasma was transferred to silicone-treated tubes for measurement of retraction. The preparation was agitated gently to assure uniform distribution of the platelets. Then to 1.0 ml. of the plasma, 0.1 ml. of a solution containing 100 units of thrombin* was added. The tubes were promptly tilted to mix the plasma and thrombin and were then left undisturbed in a water bath at 37°C. for a period of at least two hours. Clotting appeared to be complete within about 10 seconds. Retraction was usually evident in about five minutes and was complete in about 40 minutes. Clot retraction was somewhat delayed in preparations containing glycerol. Results of the clot retraction tests were recorded photographically†. A plastic test tube rack was employed to eliminate shadows. A flashed opal glass illuminator was placed about five inches behind the tubes. If the serum appeared cloudy, infra red film was used; otherwise ordinary fine grain panchromatic film was satisfactory.

The fibrinogen concentration in plasma was determined in duplicate by the method of Ratnoff and Menzie (6).

Irradiation of platelet preparations was accomplished through the use of a 250 kilovolt x-ray machine, and the unfiltered radiation was measured in air.

The glycerol diluent consisted of glycerol 36 grams, sodium chloride 0.85 grams and sufficient distilled water to bring the total volume to 100 ml. This preparation was very similar to that used by Smith (7) in studies on the preservation of red cells.

RESULTS

Normal clot retraction

Citrated platelet-rich plasma was mixed in varying proportions with platelet-free plasma from the same normal subject in order to obtain preparations with serially decreasing platelet concentrations. The plasma was then clotted by the addition of thrombin and the tubes were observed for evidence of clot retraction. The result of a typical experiment is shown in Figure 1. Platelet preparations from a large number of normal individuals have been studied by this method. Clot retraction was invariably absent when plasma had been rendered free of platelets. With a platelet concentration of 20,000 per

* Thrombin was generously supplied by Parke, Davis and Company, Detroit, Mich.

† The photographic technique was devised by Mr. Chester Reather, Photography Division, Department of Art as Applied to Medicine, the Johns Hopkins University School of Medicine.

cmm. clot retraction was always present but was incomplete. The degree of retraction increased with increasing platelet concentration. Maximal retraction often occurred when the platelet count was about 100,000 per cmm. It was apparent with this method that the degree of clot retraction was much more significantly related to the platelet concentration than was the rate at which retraction occurred. Under the conditions of these experiments retraction always occurred promptly, often beginning within five minutes. There was no detectable increase in the degree of retraction after about one hour regardless of the platelet count. In contrast to previously reported observations (8), no difference in clot retraction could be demonstrated in preparations from normal individuals of the four ABO blood groups.

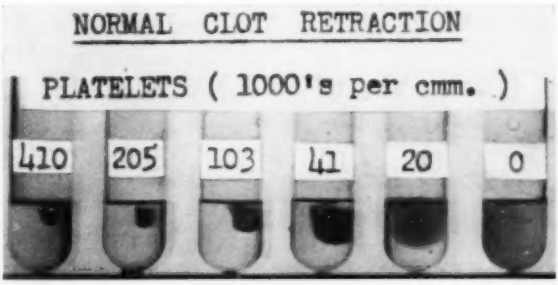


FIG. 1. The degree of retraction of plasma clots in the presence of decreasing concentrations of normal platelets is shown. The tubes are silicone-treated to prevent adherence of the clot to the container. Retraction did not increase in degree in any of the tubes after 40 minutes.

Survival of platelets in vitro *as measured by clot retraction*

Normal citrated plasma containing 300,000 platelets per cmm. was stored in silicone-treated tubes at 2°C. for varying periods up to 35 days. Periodically tubes were removed from the ice-bath, platelet counts performed and the plasma clotted by the addition of thrombin. The results of this study are shown in Figure 2. It can be seen that excellent clot retraction occurred during the first 10 days although the platelet count decreased. There was slight retraction on the 14th and 17th days, but at these times the degree of retraction was much less than would have been expected had all of the remaining platelets been normal.

Other experiments were designed to study the relative effects of glass and of silicone surfaces on the survival of platelets *in vitro*. Portions of citrated platelet-rich plasma from a normal subject were stored at 2°C. in plain glass and in silicone-treated tubes. The results of this experiment are shown in Figure 3. There is no evidence that contact with a glass surface hastened the

deterioration of platelets. The rate of decrease in platelet count was approximately the same in the two types of tubes and clot retraction was well preserved for the eight days of the experiment.

In a similar study portions of citrated platelet-rich plasma were incubated at 37°C in plain glass and in silicone-treated tubes (Fig. 4). After 24 hours retraction was not detectably impaired, but after three days retraction could not be demonstrated in plasma stored in glass or in silicone-treated tubes. The decrease in platelet count on storage was essentially the same in the two types of tubes. In other experiments the survival of platelets incubated at

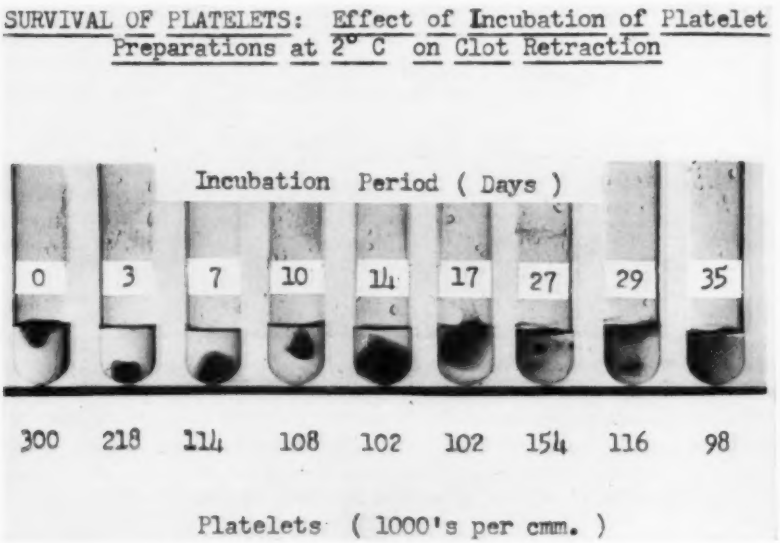


FIG. 2.

37°C. was somewhat longer, but there was again no apparent effect of the type of surface on survival time. Because of the possibility that the results of these experiments might have been influenced by bacterial contamination of the plasma, the procedure was repeated using sterile technique. Even under these conditions retraction was definitely impaired on the third day and absent on the fourth day. Daily determination of the platelet count and fibrinogen concentration showed that the platelets had decreased only from 446,000 to 342,000 per cmm. and the fibrinogen concentration from 477 mg. per 100 ml. to 326 mg. per 100 ml. at the time that impaired retraction was demonstrated.

In a more critical experiment platelet-rich and platelet-free plasma speci-

mens were prepared from the blood of a normal subject. Clot retraction was measured after appropriate mixing of the two specimens. Other portions of the same platelet-rich and platelet-free plasma were stored at 37°C. for 24 hours. Platelet counts were then repeated and the two specimens mixed in varying proportions. The degree of clot retraction in the fresh and incubated preparations is shown in Figure 5. Although the platelet count decreased

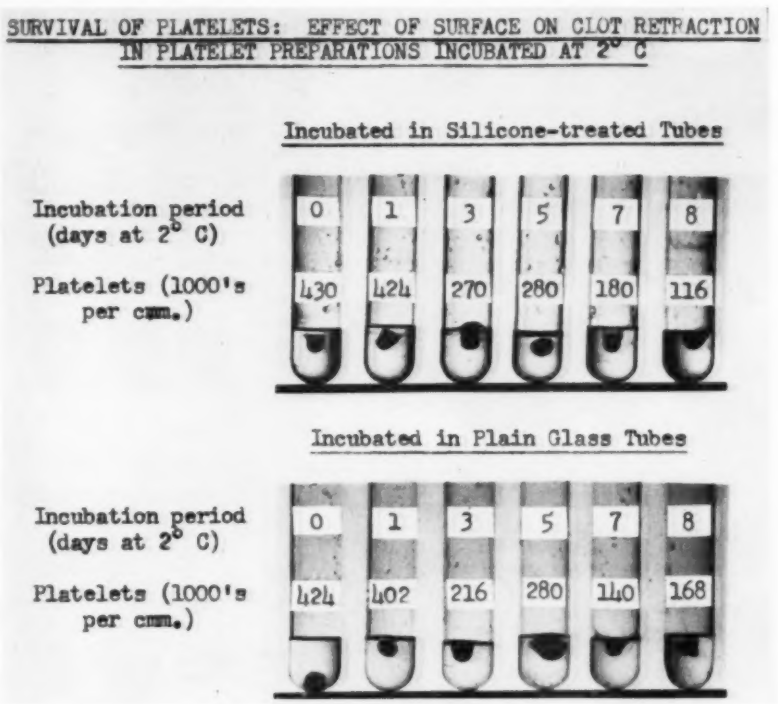

SURVIVAL OF PLATELETS: EFFECT OF SURFACE ON CLOT RETRACTION IN PLATELET PREPARATIONS INCUBATED AT 2° C
Incubated in Silicone-treated Tubes
Incubation period (days at 2° C)
0 1 3 5 7 8
Platelets (1000's per cmm.)
430 424 270 280 180 116
Incubated in Plain Glass Tubes
Incubation period (days at 2° C)
0 1 3 5 7 8
Platelets (1000's per cmm.)
424 402 216 280 140 168

FIG. 3.

from 605,000 to 506,000 per cmm. in the platelet-rich plasma during 24 hours of incubation, those platelets remaining functioned well with regard to clot retraction, appreciable retraction occurring with a platelet concentration of 25,000 per cmm.

In the studies of platelet survival which have been described, ACD solution was used as the anticoagulant. When heparin or a mixture of ammonium and potassium oxalate was employed, the ability of platelets to produce retraction disappeared much more rapidly than in control specimens in which ACD solution was the anticoagulant.

The appearance of the platelets in the stored preparations was examined using direct and phase contrast microscopy. In preparations containing ACD solution there was little evidence of clumping or agglutination of platelets. An occasional specimen showed small clumps in the counting chamber. The stored platelets sedimented but were readily resuspended by minimal agitation. The fact that clot retraction occurred in the stored plasma was in itself

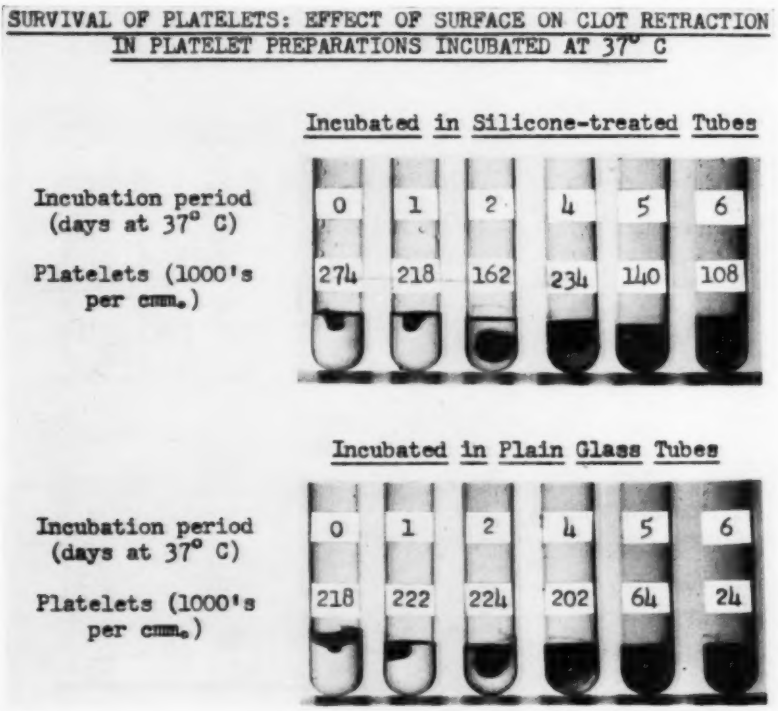
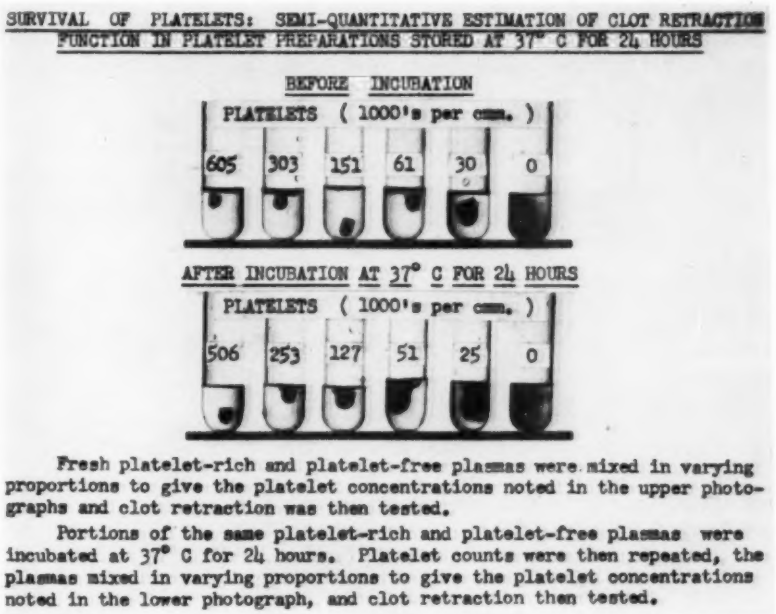

SURVIVAL OF PLATELETS: EFFECT OF SURFACE ON CLOT RETRACTION IN PLATELET PREPARATIONS INCUBATED AT 37° C
Incubated in Silicone-treated Tubes
Incubation period (days at 37° C)
0 1 2 4 5 6
Platelets (1000's per cmm.)
274 218 162 234 140 108
Incubated in Plain Glass Tubes
Incubation period (days at 37° C)
0 1 2 4 5 6
Platelets (1000's per cmm.)
218 222 224 202 64 24

FIG. 4.

evidence that the platelets had not agglutinated since it is known that agglutinated platelets do not produce retraction (9). There was little morphologic alteration in platelets stored at 2°C. On the other hand, in preparations incubated at 37°C. distinct abnormalities were apparent. The platelets appeared swollen, coarsely granular and had indistinct margins in smears stained with Wright's stain. In the experiment shown in Figure 4, these morphologic abnormalities were present at a time when clot retraction was not measurably impaired.

The fibrinogen concentration of plasma tends to decrease when the plasma is stored. In all of the experiments described, however, sufficient fibrinogen remained to form a bulky clot. Furthermore, decrease in fibrinogen concentration tends to enhance retraction rather than to impair it (10, 11). The loss of clot retraction, therefore, cannot be attributed to deterioration of the fibrinogen.



Fresh platelet-rich and platelet-free plasmas were mixed in varying proportions to give the platelet concentrations noted in the upper photographs and clot retraction was then tested.

Portions of the same platelet-rich and platelet-free plasmas were incubated at 37° C for 24 hours. Platelet counts were then repeated, the plasmas mixed in varying proportions to give the platelet concentrations noted in the lower photograph, and clot retraction then tested.

FIG. 5.

Effect of red cell agglutinins on clot retraction

In certain pathologic states platelet agglutinins have been demonstrated in plasma (12, 13, 14). In the presence of these agglutinins platelets fail to produce clot retraction. In the present study a variety of red cell agglutinins were tested for their ability to agglutinate platelets and to influence retraction. Citrated platelet-free plasma specimens containing various red cell agglutinins in high titer were mixed with platelet-rich plasma from an individual whose blood contained the corresponding red cell agglutinogens. The results of the experiment are shown in Figure 6. There was no agglutination of platelets and no interference with clot retraction by any of the red cell agglutinins tested.

Effect of ionizing radiation on the clot retraction function of platelets

Werner (15) has reported that direct exposure of platelets to large amounts of x-ray (1200 r) led to impairment of clot retraction. In the present experiments citrated platelet-rich plasma was transferred to silicone-treated Petri dishes so that the plasma could be spread in a thin film with a depth of only a few millimeters. The material was then exposed directly to 10,000 roentgens

THE EFFECT OF RED CELL AGGLUTININS ON CLOT RETRACTION

Controls:
1 = Normal platelet-rich plasma without antibody.
2 = Normal platelet-rich to which was added a human serum that agglutinates human platelets.

Antibody Titres of Red Cell Agglutinins:

	Albumin	Serum	Saline
Anti-D	12,288	384	96
Anti-D	3,072	12	negative
Anti-M	128	384	96
Anti-A		1536	768
("Immune titre"*)		768	24
Anti-B		6144	1536
("Immune titre"*)		3072	96

* After the addition of A and B substance.

FIG. 6. The normal platelet-rich plasma was obtained from an individual of blood type A_1B M Rh_1 Hr′ negative. The control serum which agglutinated platelets and abolished clot retraction was kindly provided by Dr. Mario Stefanini.

of x-ray. Sufficient back-scatter was created to assure that the platelets received a very large quantity of ionizing radiation. Another portion of the plasma was handled in a similar manner but was not irradiated. Platelet counts were performed and clot retraction tested over a period of 15 days. Ionizing radiation in this extremely high concentration did not abolish clot retraction. Furthermore, the survival of the treated platelets *in vitro* was not significantly different from that of the control. Clot retraction was demonstrable in the irradiated plasma even after 15 days storage at 2°C.

Protection of platelets by glycerol during freezing

Rapid freezing and thawing of platelet preparations abolished clot retraction. Silicone-treated tubes containing platelet-rich plasma were immersed in a mixture of ethyl alcohol and dry ice. The plasma was subsequently thawed in a water bath at 37°C. Platelet preparations treated in this manner produced no clot retraction when the plasma was subsequently clotted with

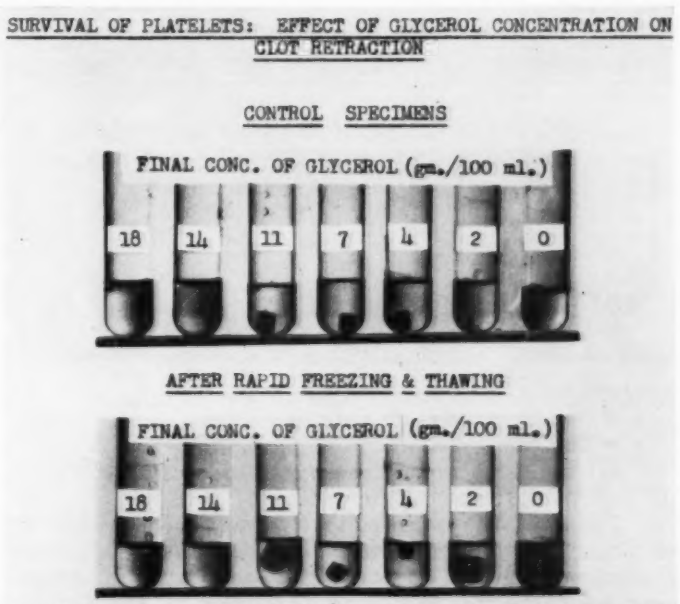


FIG. 7.

thrombin. Platelet counts were decreased by about 50 per cent after freezing, and platelets appeared badly damaged in smears stained with Wright's stain.

Smith (7) has demonstrated that human red cells can be preserved by quick freezing and thawing in the presence of 10 to 20 per cent glycerol. In the present experiments the addition of a glycerol diluent to platelet-rich plasma prevented the loss of clot retraction function after freezing. Figure 7 shows the results of an experiment in which the final concentration of glycerol varied over a wide range. When the final concentration of glycerol was relatively high, coagulation was incomplete, glycerol appearing to retard the clotting of

plasma by thrombin. The concentration of glycerol which seemed optimal for preservation of clot retraction function was four to seven per cent. Platelet counts decreased by about 25 per cent after freezing and thawing in the presence of glycerol. The remaining platelets appeared normal when examined microscopically in unstained preparations. Glycerol prevented adequate staining of platelets with Wright's stain, so that comparisons before and after freezing were not satisfactory.

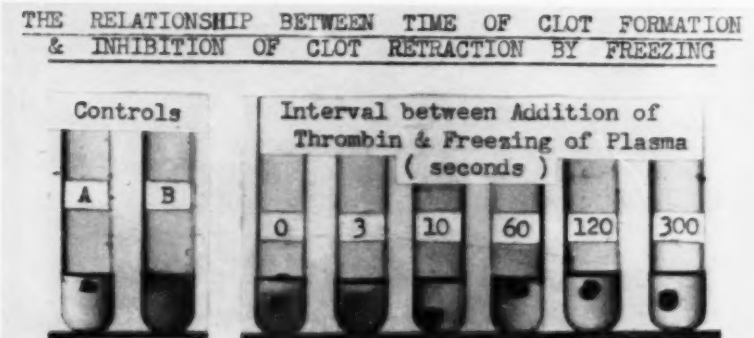

THE RELATIONSHIP BETWEEN TIME OF CLOT FORMATION & INHIBITION OF CLOT RETRACTION BY FREEZING
Controls
A
B
Interval between Addition of Thrombin & Freezing of Plasma (seconds)
0
3
10
60
120
300
Controls:
A = The contents of this tube were not frozen.
B = The contents of this tube were frozen & thawed before the addition of thrombin.
The platelet-rich plasma in all tubes clotted in less than three seconds after the addition of thrombin. Thrombin was added to the third tube at the precise moment of freezing. Only in the tube on the far right had clot retraction started before the contents were frozen.

FIG. 8.

Failure of freezing to inhibit retraction when the clot is formed prior to freezing

As demonstrated in previous experiments, freezing of platelet-rich plasma prior to the addition of thrombin abolished subsequent clot retraction, presumably by disruption of platelets. In other experiments, platelet-rich plasma was frozen after coagulation had been initiated by the addition of thrombin. Figure 8 shows the results of a typical study. When the preparation was frozen after the addition of thrombin in high concentration, retraction function was abolished provided that coagulation had not occurred at the time of freezing. However, if freezing was delayed until clotting was evident, then

clot retraction was unimpaired after thawing. When a potent preparation of thrombin was used, as in the experiment described in Figure 8, retraction could not be impaired by freezing the preparation 10 seconds after the addition of thrombin, for at this time coagulation had already occurred.

DISCUSSION

There is currently great interest in developing methods for concentrating, storing and transfusing blood platelets (16–19). Platelets are fragile structures and may easily be damaged by the manipulations required in these procedures. It is difficult to determine the viability of platelets after manipulation *in vitro*. One can observe their hemostatic effect and can measure their survival time *in vivo* after transfusion of the platelets to a thrombocytopenic recipient. However, this procedure is fraught with difficulties because of the variable responses of the recipient including the possible development of antibodies against the transfused platelets. Unfortunately, there is no certain way of determining the integrity of platelets *in vitro*. The concentration of platelets can be determined, their morphology studied microscopically, their clot-accelerating activity measured, their ability to produce clot-retraction estimated and their vasoconstrictor activity assayed. There is clear evidence that no one of these determinations is adequate to reveal whether platelets are viable and capable of exerting their normal hemostatic effect. Some investigators interested in platelet preservation have attempted to use clot-accelerating activity as a measure of the viability of platelets in stored preparations (18). This criterion is obviously unsatisfactory. It is well known that macerated platelets or even platelet extracts may have potent clot-accelerating effects *in vitro* although these preparations have no hemostatic value *in vivo*. Furthermore, the clot-accelerating function of platelets is one of their more stable properties and persists long after functions more sensitive to injury have been lost.

On the other hand, ability to produce clot retraction depends upon the presence of intact platelets. The occurrence of clot retraction in a platelet preparation does not prove that these platelets would have functioned normally *in vivo*. It is known that in certain disorders hemorrhagic manifestations can be attributed to platelet abnormalities even though clot retraction is normal (20). However, the inability of platelets to produce clot retraction is clear evidence of their abnormality and probable functional inadequacy.

There is general agreement that platelets degenerate rapidly in stored blood (21–29). In some of these studies very rapid disappearance of platelets has been reported, only about 25 per cent remaining after the third day. On the other hand Copley and Houlihan (30) found as many as 80 per cent present after 35 days in platelet suspensions prepared from heparinized or citrated blood. Few studies concerning the function of those platelets remaining have

been carried out. The criteria for platelet survival in the studies mentioned were based on estimation of the number of platelets remaining and in some instances on their morphologic appearance. It is of interest that Kolmer (26) in 1940 noted that platelet survival was greatly improved when dextrose was incorporated into the citrate anticoagulant mixture.

Lundsteen (31) observed that when platelet-rich plasma containing sodium citrate was stored at 37°C., retraction was considerably impaired within 8 hours and was absent after 24 hours although the number of platelets which could be counted had not decreased. On the other hand there was only slight loss of retraction after 24 hours storage at room temperature or in the refrigerator.

In the present studies ACD solution was used as the anticoagulant. It is noteworthy that clot retraction could be demonstrated in platelet-rich plasma after storage for two weeks at 2°C. even though there was a progressive decrease in platelet concentration during this period. However, on prolonged storage of plasma recognizable platelets as well as adequate fibrinogen persisted after clot retraction no longer occurred. Even at 37°C. platelet counts were well maintained for 24 to 48 hours and clot retraction remained good.

Much attention has been devoted to the effect of various surfaces on the stability of the platelets. When blood is undergoing coagulation remarkable morphological changes take place in the platelets. If the blood is not protected from clotting by the addition of anticoagulant, the type of surface is of great importance in determining the rate at which these platelet changes occur. A number of investigators believe that a wettable surface is damaging to platelets even when blood is prevented from clotting by an anticoagulant. This view stems in part from the belief that coagulation is initiated by the effect of wettable surfaces on platelets. It is entirely possible, however, that the changes which platelets undergo are secondary to an alteration in plasma produced by contact with a wettable surface (32). In the present investigation in which anticoagulant was employed, platelets showed no greater tendency to clump, to adhere to the walls of the container, to decrease in number, or to lose clot retraction function when stored in plain glass tubes than when stored in silicone-treated tubes.

It has recently been reported that there may be platelet antigens comparable to but differing from the red cell antigens. In view of the interest in this problem an effort was made to determine whether platelets can be affected by red cell agglutinins. Our results are in agreement with the observations of Harrington (12) and Stefanini (13) that the various red cell antigens are not present in the platelets.

It has been repeatedly demonstrated that platelets lose their ability to produce clot retraction after freezing and thawing (11). The addition of glycerol to platelet-rich plasma exerted a remarkable protective action. The con-

centration of glycerol which was optimally effective appeared to be lower than that recommended by Smith (7) for the protection of red cells during freezing.

One of the most fascinating observations which was made during the present study concerned the effect on clot retraction of freezing plasma after thrombin had been added. When a platelet preparation was frozen and thawed after clot formation had occurred but before retraction had begun, there was no interference with ultimate clot retraction. Thus, within 10 seconds after the addition of a potent thrombin preparation, retraction function could no longer be abolished by freezing. Presumably the abolition of clot retraction by prior freezing is the result of disruption of platelets. The manner in which clot formation prevents this effect is unknown. It is possible that clot formation may exert a protective action on platelets during freezing in a manner similar to the protection afforded by glycerol. On the other hand it is possible that by the time clot formation occurs the platelets have already set into motion those forces which lead to ultimate retraction of the clot. If the latter explanation is correct, then the changes produced by the platelets to bring about ultimate clot retraction take place in a remarkably short period of time and are perhaps related to the manner of arrangement of fibrin strands.

SUMMARY

Survival of blood platelets *in vitro* under various experimental conditions was studied by a method for semi-quantitative measurement of clot retraction. Evaluation of clot retraction function was considered to be a more reliable measure of platelet survival than other methods because retraction does not occur unless platelets are intact. When platelet-rich plasma containing ACD solution was stored at 2°C., there was progressive decrease in platelet count, but ability to produce clot retraction was lost more rapidly than could be accounted for by numerical decrease in platelets. Nevertheless, retraction could be detected after storage for periods longer than two weeks. Deterioration of platelets occurred much more rapidly at 37°C. In the presence of the anticoagulant, glass surfaces did not hasten dissolution of platelets as compared to silicone-treated surfaces. Platelets were not agglutinated or destroyed, and retraction function was not impaired by red cell agglutinins in high titer or by heavy exposure to ionizing radiation. The ability of platelets to produce clot retraction was totally destroyed by freezing and thawing, provided that clot formation had not occurred at the time of freezing. In the presence of glycerol in appropriate concentration, platelets were protected against this destruction by freezing.

We should like to express our sincere appreciation to Miss Elsa Jahn, Baltimore Rh Typing Laboratory, who referred to us the individuals with red cell agglutinins in high titer, and who determined the titer of these agglutinins.

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CLOT RETRACTION AS A MEASURE OF PLATELET FUNCTION[1]

II. Clinical Disorders Associated with Qualitative Platelet Defects (Thrombocytopathic Purpura)

DUDLEY P. JACKSON,[2] ROBERT C. HARTMANN, AND C. LOCKARD CONLEY

Department of Medicine, the Johns Hopkins University School of Medicine and Hospital

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In the preceding report (1), a technique for the study of the clot retraction function of platelets has been described. This method, together with other techniques, has been used to study platelet function in patients with certain hemorrhagic diseases. The results indicate that abnormal bleeding may occur in association with certain qualitative defects of platelet function even though the number of platelets is not reduced.

METHODS

Blood specimens were obtained from the patients' antecubital veins, usually through an 18 gauge needle which had not been treated with a non-wetting agent. All syringes were treated with silicone,[3] and most also were lubricated with silicone oil.[4] Platelets were counted by the phase contrast technique of Brecher and Cronkite (2) in which 1 per cent ammonium oxalate is used as the diluent. A few of the earlier counts (Cases # 1, 2, and 3) were performed in duplicate by a direct method using Rees-Ecker diluent but subsequent counts on these patients were determined by the phase contrast technique. *Bleeding times* were determined by the Duke technique using the ear lobe. In normal individuals the bleeding time usually does not exceed six minutes. *Tests for capillary resistance* were performed using a sphygmomanometer set at 80 mm. Hg for 5 minutes. The arm distal to the cuff was observed for the presence of petechiae immediately and 10 minutes after the removal of the cuff. *Whole blood clotting times* were determined as follows: 1.0 ml. of whole blood was added directly from the syringe to each of 3 chemically clean, dry, 13 x 100 mm. Pyrex test tubes immersed in a water bath at 37°C. The stop watch was started at the time that blood was first seen in the syringe. At the end of 4 minutes, the first tube was observed for evidence of clotting by gentle tilting at approximately one minute intervals. After the first tube had clotted, the second tube was observed in a similar manner, and finally the third tube. The end point of clotting was that time at which the tube could be inverted with no flow. Only the clotting time of the third tube is recorded. The normal range is approximately 15 to 30 minutes. *Whole blood clot retraction tests* were performed in duplicate using a semiquantitative technique as follows: 3.0 ml. of blood were added directly from the syringe to a 13 x 100 mm. silicone-treated test

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[2] Postdoctorate Research Fellow, U. S. Public Health Service.

[3] General Electric Dri-film SC-87 (formerly 9987).

[4] General Electric Silicone Oil SF-96 (200) (formerly 9996-200).

tube containing 0.1 ml. of thromboplastin prepared from rabbit brain. A paper clip was suspended in the lower third of the tube prior to the introduction of the blood. The tube was left undisturbed, and was incubated for 1 hour at 37°C. At the end of the incubation, the paper clip, with the adherent and retracted clot, was removed and discarded. The remaining cells and serum were centrifuged to determine the volume of serum expressed. The amount of clot retraction was calculated as follows: $\frac{\text{Residual Serum}}{(3 - \text{V.P.C.}^5) + 0.1} \times 100 =$ per cent residual serum (i.e. per cent clot retraction). Normally this value should be 75 per cent or more. *Clot retraction in a plasma system* was measured by the method described in the preceding report (1). *Prothrombin times* were determined in duplicate at 37°C on a mixture of 0.1 ml. of the patient's oxalated plasma, 0.1 ml. of a standardized rabbit brain thromboplastin, and 0.1 ml. of M/40 calcium chloride solution. The normal range is 16–18 seconds. *Prothrombin consumption by the one-stage procedure* was measured using a modification of the method of Quick and Favre-Gilly (3). This test was performed in duplicate. In this procedure, 2.0 ml. blood in a 13 x 100 mm. chemically clean, Pyrex test tube were incubated at 37°C for one hour from the time of obtaining the blood. At the end of the incubation, clotting was stopped by the addition of sodium oxalate, the clot was broken, the tubes were shaken, and then centrifuged. After centrifugation, the tubes were returned to the 37°C water bath for an additional 30 minutes. At the end of this time, the residual prothrombin was determined in duplicate by the one-stage procedure using a mixture containing 0.1 ml. of the serum-plasma, 0.1 ml. of a standardized rabbit brain thromboplastin, 0.1 ml. of barium sulfate adsorbed plasma from a normal individual and 0.1 ml. of M/40 calcium chloride solution. *Prothrombin consumption by the two-stage procedure* was measured using a modification of the method of Buckwalter, Blythe, and Brinkhous (4). In this procedure, 2.0 ml. of blood were added to each of 4 chemically clean, 13 x 100 mm., Pyrex test tubes, and the tubes were incubated at 37°C. After 15 minutes, sodium citrate was added to the first tube, the clot was broken and the tubes shaken. The tube was centrifuged and the residual prothrombin concentration was determined by the two-stage procedure of Ware and Seegers (5). A similar procedure was carried out at 30, 45, and 60 minute intervals in the next 3 tubes. *Fibrinogen* determinations were performed by the technique of Ratnoff and Menzie (6).

RESULTS

Platelet Function in Thrombocytopathic Purpura

Case 1: E. T. (JHH #637682), a 12 year old colored male was referred to the Johns Hopkins Hospital with a tentative diagnosis of hemophilia. There was no family history of abnormal bleeding. The patient's mother noted that when the patient first began to walk there was a tendency to easy bruising. The patient had frequent nose bleeds, some of which were very severe. Excessive bleeding followed dental extractions on several occasions. At the age of 7 years the patient was subjected to tonsillectomy and adenoidectomy, and circumcision with excessive bleeding following these procedures. It was at this time that a diagnosis of hemophilia was suggested. However, the patient has never had evidence of bleeding into the joints, and he has never received a blood transfusion. About three months prior to admission, the patient developed edema of the face, ankles, and genitalia. At this time he passed grossly bloody urine for a period of one week. He was considered to have acute glomerulonephritis. At the time of admission there was residual evidence of nephritis in that there were a few red blood cells and a small amount of albumin in the urine. Subsequently

[5] V.P.C. The volume of packed red blood cells of the original 3.0 ml. of blood.

these abnormalities completely cleared. The patient appeared to be normally developed and well nourished. He was afebrile and the blood pressure was normal. There was slight bleeding from the gums, but no other evidence of bleeding. No petechiae were noted. The physical examination otherwise was not remarkable. There was no lymphadenopathy, and the liver and spleen were not enlarged. Since discharge from the hospital the patient has been in excellent general health, though he has had persistent mild bleeding from the gums and there has been one mild epistaxis. Care has been taken to avoid injuries or vigorous athletics. The serological test for syphilis was negative. Volume of packed red blood cells was 30 per cent following the episode of nephritis but subsequently rose to 38.4 per cent. Repeated white blood cell counts were within the normal range and the differential count has been unremarkable. Renal function studies revealed no persistent abnormality and blood chemical determinations were normal. The serum calcium was 11.8 mgm. per 100 ml.

A number of special studies were performed to determine the nature of the hemostatic defect. The whole blood clotting time was 24 minutes. Prothrombin concentration was normal when measured by the one and two-stage procedures. On multiple determinations the platelet count ranged between 346,000 and 455,000 per cmm. The rate of conversion of prothrombin to thrombin (prothrombin consumption) during clotting was measured by the one and two-stage procedures. The results were normal in both instances. Using the two-stage procedure, 75 per cent of the prothrombin was utilized at the end of 45 minutes, and 84 per cent utilized at the end of 60 minutes. Plasma fibrinogen was 312 mgm. per 100 ml., and there was no lysis of the sterile recalcified plasma clot after 48 hours incubation at 37°C. Test of capillary resistance was positive (abnormal). On each of 4 determinations, the bleeding time was greater than 20 minutes. Blood clots were well formed but showed no evidence of clot retraction.

Platelet function as measured by clot retraction of a plasma system was determined by the method previously described (1). Figure 1 presents these results. It is apparent that this patient's platelets produced no clot retraction even at a concentration of 400,000 platelets per cmm. This retraction defect might have been due either to a platelet or to a plasma abnormality. In order to study these possibilities, other experiments were performed. Normal platelet-rich plasma was serially diluted with platelet-free plasma from the patient. Eighteen thousand normal platelets per cmm. in a mixture containing 95 per cent of the patient's platelet-free plasma produced good retraction. It is evident that the patient's platelet-free plasma did not inhibit clot retraction by normal platelets. When platelet-rich plasma from the patient and from a normal individual were mixed in various proportions, the degree of clot retraction was related to the concentration of normal platelets. Furthermore, when the patient's platelet-rich plasma was mixed with normal platelet-free plasma, no retraction occurred, even in a mixture containing 207,000 platelets from the patient and 50 per cent normal platelet-free plasma. These studies demonstrate conclusively that the defect of clot retraction was caused by a platelet abnormality.

The morphology of the patient's platelets was studied by direct and phase contrast microscopy. The patient's platelets appeared perfectly normal on smears stained with Wright's stain and in fresh preparations studied under the phase contrast microscope. Preparations of the patient's platelet-rich plasma containing acid citrate dextrose (ACD) were recalcified, and the clotting process observed under the phase microscope. As coagulation took place, the patient's platelets were observed to undergo the same changes as are seen in normal

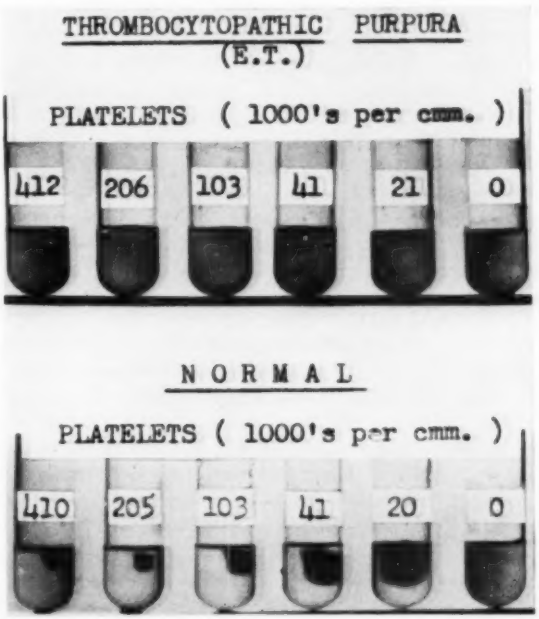

FIG. 1. Clot retraction studies in Case 1 (E. T.). Platelet-rich plasma from the patient was serially diluted with platelet-free plasma from the same source. The preparations were clotted by the addition of thrombin. No retraction occurred at any platelet level tested in contrast to the normal control shown below.

platelets under the same conditions. Long filamentous processes were seen to extend from the platelets and bulbous enlargements developed at the bases of these processes in a manner indistinguishable from the normal. Strikingly different from the normal was the failure of the fibrin strands to become oriented around the platelets. Fibrin formation appeared to occur without relation to the platelets, whereas in normal preparations, fibrin strands were rapidly oriented in such a way that they seemed to radiate out from the platelets. Bone marrow aspirated from the sternum of this patient contained numerous megakaryocytes indistinguishable from normal megakaryocytes.

The patient's mother, father, and 10 year old brother were studied and all had normal prothrombin times, platelet counts, and clot retraction. Clotting times done on the parents were normal. The mother had a normal test of capillary resistance, and a bleeding time of 8½ minutes. The father's blood revealed a normal rate of conversion of prothrombin to thrombin. One brother and two sisters have not yet been studied.

Case 2: B. C. (JHH #A 85528), a 7 year old colored female was admitted with a chief complaint of easy bruising and frequent nose bleeds of 4 years duration. There was no family history of abnormal bleeding. There was a history of 4 episodes of pyelitis manifested by fever, abdominal pain, nausea, anorexia, and pyuria. These episodes were treated with penicillin, and there have been no apparent sequelae. Since the age of 3 years, the patient has experienced repeated episodes of easy bruising, spontaneous purpura, epistaxes, and bleeding from the gums. On two occasions she received blood transfusions. She was admitted to the Johns Hopkins Hospital for study of her hemorrhagic disorder. The child was a normally developed and well nourished young colored female. She was afebrile, and the blood pressure was normal. There were many purpuric spots, some with a diameter as great as 5 mm. over the trunk and extremities, and there were several small petechiae on the chest. No hemorrhagic lesions were noted on the mucous membranes, and there was no bleeding from the gums. There was no significant lymphadenopathy, and the liver and spleen were not enlarged. Otherwise the physical examination was not remarkable. The serologic test for syphilis was negative. Volume of packed red blood cells was 37 per cent. The white blood cell count was 9,000 per cmm. and the differential count revealed 1 per cent neutrophilic metamyelocytes, 30 per cent polymorphonuclear leucocytes, 3 per cent eosinophilic metamyelocytes, 20 per cent eosinophils, 43 per cent lymphocytes, and 3 per cent monocytes. Direct eosinophil count was 2,000 per cmm. Renal function studies revealed no persistent abnormality. Blood chemical determinations were normal and the serum calcium was 11.5 mgm. per 100 ml. X-rays of the skull and long bones, and intravenous pyelogram were normal. No ova or parasites were demonstrated in the stool. Serum iron concentration was 45 micrograms per 100 ml. No. L.E. cells were demonstrated in the buffy coat of the peripheral blood.

A number of special studies were performed to determine the nature of the hemostatic defect. The whole blood clotting time was 23 minutes. Prothrombin concentration was normal by the one-stage procedure (17 and 19 sec.). Two platelet counts were 220,000 and 242,000 per cmm. respectively. The rate of conversion of prothrombin to thrombin (prothrombin consumption) during clotting was normal by the one-stage procedure. The test of capillary resistance showed a striking increase of capillary fragility on 2 occasions. On 4 determinations the bleeding time was >25, 12, >20, and >14 minutes. Whole blood clot retraction was markedly impaired (30 per cent).

Platelet function as measured by clot retraction of a plasma system was determined by the method described. Figure 2 presents the results. It is apparent that although there was a fair degree of clot retraction when the platelets were concentrated to a level of 400,000 per cmm., at lesser platelet levels (200,000 per cmm. or less) there was impairment of retraction as compared to that of

the normal control. On the stained smear the platelets were bizarre, coarsely granular, and irregular in size, many appearing quite large (Fig. 3).

Bone marrow aspirated from the sternum of this patient contained megakaryocytes which were indistinguishable from the normal. Eight per cent of the nucleated cells in the marrow were eosinophils.

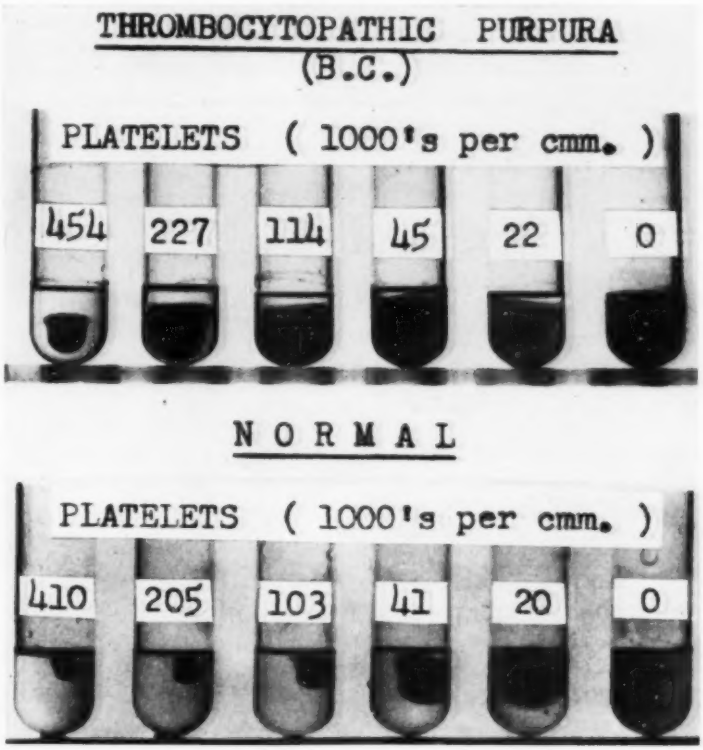

FIG. 2. Clot retraction studies in Case 2 (B. C.). Plasmas with varying platelet levels were prepared as in Figure 1. Clot retraction occurred in this case but was less in degree at each platelet level than in the normal control.

Studies performed on the patient's mother revealed normal whole blood clotting time, platelet count, prothrombin time, bleeding time, test of capillary resistance, whole blood clot retraction (80 per cent), and prothrombin consumption by the one-stage technique. The mother's platelets appeared normal on smears stained with Wright's stain.

Case 3: J. M. (JHH #568110), a 5 year old white male has been admitted to the Johns Hopkins Hospital on 6 occasions since 1951 because of a hemorrhagic disorder which had

been evident since early life. There was no family history of abnormal bleeding. The patient has been noted to bruise easily. He has bled profusely from minor cuts and scratches, and prolonged pressure was required to cause hemorrhage to cease. At the age of 1½ years he had an exsanguinating hemorrhage from the nose, and since that time severe epistaxes have occurred at frequent intervals. He has had numerous transfusions following hemorrhage. He has never been noted to have evidence of bleeding into muscles or joints. Tonsillectomy was

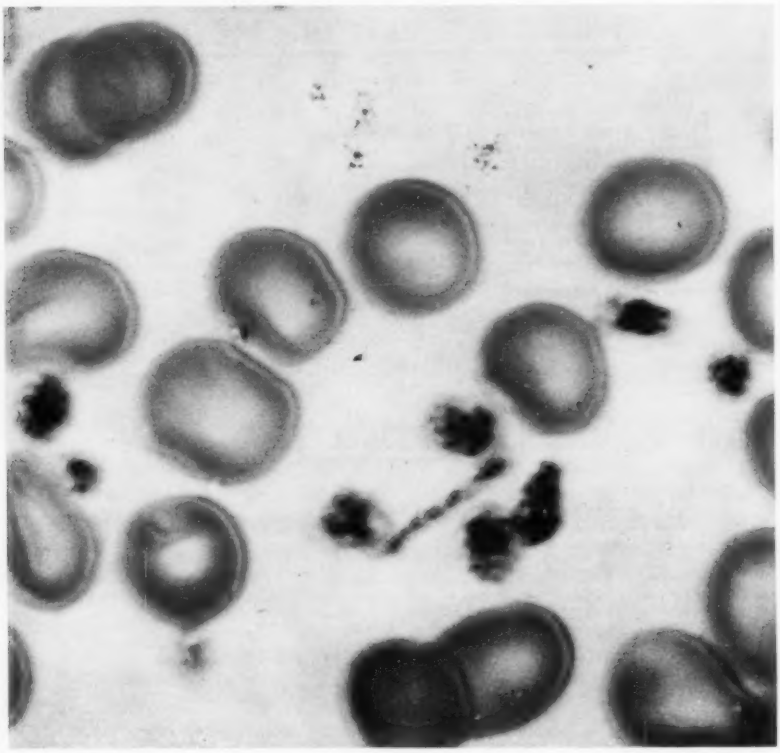

FIG. 3. Photomicrograph of blood smear from Case 2 (B. C.) showing abnormal platelets. Smear stained with Wright's stain. 1250 X.

performed at another hospital when the patient was 2 years of age. Bleeding from the operative site occurred on the first post-operative day but was of minor degree. Admissions to the Johns Hopkins Hospital have been for the most part occasioned by severe hemorrhages from the nose, requiring repeated transfusions. Profound anemia was present at these times, but there was no anemia at times remote from hemorrhage. On examination the patient was a well nourished youngster of normal growth and development. Pallor was marked following nasal hemorrhage. Small ecchymoses were seen in the skin but there was no other evidence of abnormal bleeding except from the nose. There was no adenopathy, and the liver and spleen were not palpable. Bleeding from the nose was at times difficult to control, requiring packing for several days and repeated blood transfusions. Many laboratory tests were performed,

and the studies to be recorded below indicated that the patient had platelets which were morphologically and functionally abnormal. The white blood cell count was occasionally elevated following the episodes of bleeding, but on most occasions was normal. The differential count was normal except that on several occasions there was an eosinophilia (maximum 8 per cent). Numerous blood chemical tests were within normal limits. Platelet counts were performed on many occasions. The platelet counts were low, ranging between 66,000 and 140,000 per cmm. However, on several occasions, severe epistaxes occurred at a time when the platelet count was greater than 100,000 per cmm.

Because of the persistent thrombocytopenia, it was decided to perform a splenectomy even though it was realized that there was an associated abnormality of the function of the platelets. The splenectomy was performed in July 1952, and there was no excessive bleeding. The spleen weighed 66 grams, and on section appeared normal except for some hyperplasia of the lymphoid follicles. The post-operative course was satisfactory. Following the splenectomy the platelet count slowly rose to normal levels and has been maintained at approximately 300,000 per cmm. since the operation. However, there has been no improvement in the hemorrhagic disorder. The patient has continued to have severe epistaxes, requiring 3 hospital admissions for transfusions since the operation.

Special studies were performed prior to the splenectomy in order to determine the nature of the hemorrhagic disorder. Whole blood clotting time was normal on repeated occasions, ranging between 11 and 20 minutes. Prothrombin time by the one-stage method was repeatedly normal. The test of capillary resistance was distinctly positive on numerous occasions. The whole blood clot retraction was normal (91 per cent) at a time when the platelet count was 101,000 per cmm. Clot retraction was measured on a plasma system by the method described and was normal, as shown in Figure 4. The bleeding time was determined on many occasions and was prolonged, at times exceeding 20 minutes. The bleeding time was greater than 20 minutes when the platelet count was 140,000 per cmm. Prothrombin consumption by the one-stage procedure was abnormal prior to splenectomy. At a time when the platelet count was 101,000 per cmm., little or no prothrombin was utilized during clotting. The platelets were abnormal on smears stained with Wright's stain, with large, bizarre, irregular, and occasional bacilliform forms present (Fig. 5). Aspiration of bone marrow was performed on two occasions and megakaryocytes appeared normal. Following splenectomy, even though the platelet count rose to levels in excess of 300,000 per cmm., there was no improvement in prothrombin consumption as measured by the one-stage technique.

Examination of the mother revealed platelets of normal number and morphology, and the bleeding time and prothrombin consumption tests were normal. Examination of the father revealed normal bleeding time, tourniquet test, platelet count, whole blood clotting time and prothrombin consumption. The smears of the father's blood stained with Wright's stain revealed a moderate number of bizarre platelets. The patient has two siblings. One six year old sister has no history of abnormal bleeding and platelets are said to be normal

on smear. The other sibling, a one year old sister, has a platelet count of 194,000 per cmm., and on smears stained with Wright's stain many of these platelets are of bizarre size and shape and appear abnormal. This child has had a single episode of mild, spontaneous epistaxis.

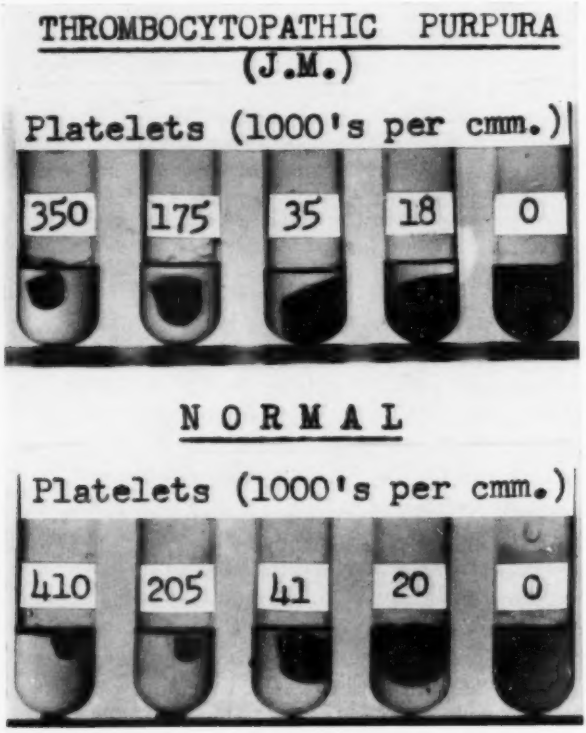

FIG. 4. Clot retraction studies in Case 3 (J. M.). Plasmas with varying platelet levels were prepared as in Figure 1. Clot retraction in this case was not significantly different from the normal control.

Case 4: N. C. (JHH #619181), a 43 year old white female was referred to the Johns Hopkins Hospital for study of a hemorrhagic disorder. The family history was of great interest. There was no history of abnormal bleeding on the maternal side of the family. The patient's sister has an identical hemorrhagic diathesis and will be presented as Case 5. The patient's brother has had frequent episodes of spontaneous epistaxis and required 15 blood transfusions for serious bleeding following a fractured jaw. This brother has a son who bruises easily and has had prolonged bleeding from minor lacerations. The patient's father has always bruised easily, bled freely from minor lacerations and had severe epistaxes, some of which have been followed by syncope. However, he has never received a blood transfusion. The patient's first cousin (i.e. her father's sister's son) has had frequent profuse bleeding from

the nose. Details concerning the histories of the father's thirteen other siblings are not well known. There is no known family history of hemarthrosis.

Beginning in childhood, the patient experienced easy bruising and excessive bleeding from minor injuries. During adolescence she had occasional mild epistaxes, bleeding from the gums, and was found to be anemic on several occasions. Menarche was at the age of 12. Menses were regular but the bleeding was excessive. In 1945, at the age of 36, the patient

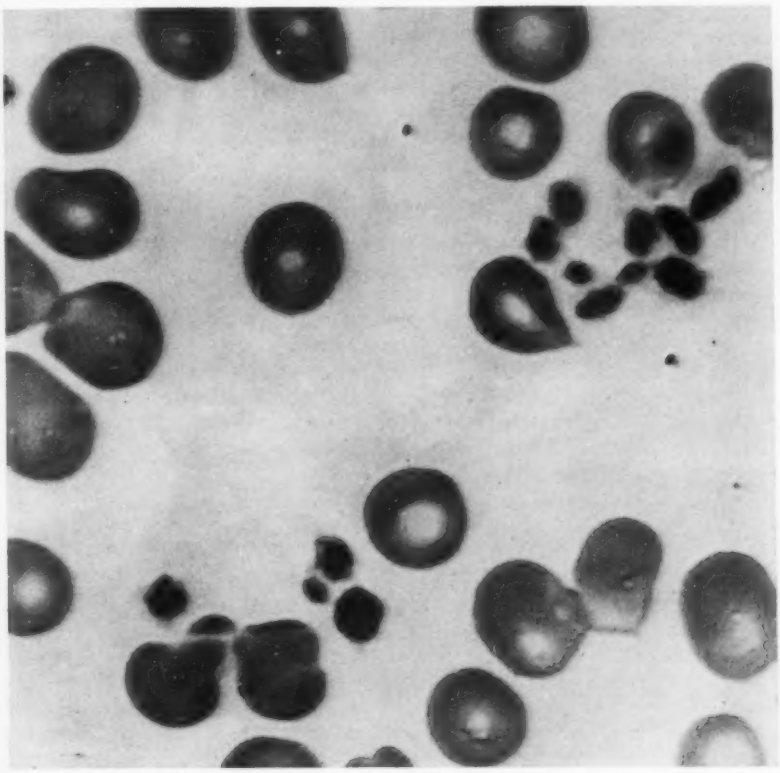

FIG. 5. Photomicrograph of blood smear from Case 3 (J. M.) prepared prior to splenectomy. Abnormal platelet forms are apparent. Smear stained with Wright's stain. 1250 X.

experienced her first episode of gastrointestinal bleeding associated with melena, weakness, palpitations and syncope for which she received three blood transfusions. Between 1945 and 1948 she had three or four such episodes, each requiring hospitalization and blood transfusions. Some of these episodes were preceded by mid-epigastric gnawing pain relieved within 10 to 20 minutes by milk or amphogel. However, on two occasions both gastro-intestinal X-ray studies and gastroscopic examinations were normal. In 1948 exploratory laparotomy failed to reveal a localized bleeding site. Three months later after another episode of melena, a splenectomy was performed because of "slightly low platelets". After each of the opera-

tive procedures, the patient bled excessively into the abdomen and subcutaneous tissues. Since splenectomy the patient has had seven or eight episodes of melena. Easy bruising, epistaxes, and bleeding from the gums have continued unabated, but the menstrual flow has no longer been excessive.

Since 1945 the patient has received at least 60 blood transfusions. During this time the only reported hemostatic abnormality has been prolonged bleeding time and slightly decreased platelet concentration. At no time has there been hematuria, hemoptysis, hematemesis, or bleeding into the joints.

The patient appeared in good general health, and the physical examination was unremarkable except for a 10 cm. ecchymosis on the left thigh. There were no petechiae or telangiectases. There was no lymphadenopathy and the liver could not be felt. The joints appeared perfectly normal. The blood pressure was normal. There was no anemia at the time of examination and the volume of packed red cells was 45.0 per cent.

The whole blood clotting time on two determinations was 37 and 34 minutes. One-stage prothrombin time was 19 and 20 seconds on two determinations. Platelet counts were 159,000 and 188,000 per cmm. Test of capillary resistance was normal. Whole blood clot retraction was normal (89 per cent) and clot retraction measured on a plasma system was normal. The bleeding time was greater than 20 minutes. The patient's conjunctival vessels were studied directly using the corneal microscope. The conjunctival vessels appeared somewhat tortuous, but no really significant abnormality could be made out. Individual vessels were traumatized under direct observation. A non-lacerating injury was found to be followed by sustained constriction of the vessels. There was thus no evidence that the vessels could not contract normally. When the vessel was lacerated, bleeding was somewhat more severe than in normal individuals. One-stage prothrombin consumption tests gave abnormal results on repeated occasions. There was no demonstrable prothrombin utilization. The platelets appeared abnormal on smears stained with Wright's stain. They were large, varied in size and were irregular in shape.

Case 5: M. C. (JHH #619172), a 41 year old white female was the sister of the patient described as Case 4, and the interesting family history has been recorded. Since early childhood the patient has experienced easy bruising, excessive bleeding from minor lacerations, and moderately severe epistaxes. In 1932 at the age of 21 she bled excessively from the wound after appendectomy and was given one blood transfusion. In 1939 she had one bout of unexplained hematuria, for which she was hospitalized but not transfused. One dental extraction in 1944 was not accompanied by abnormal bleeding.

In August 1952 without preceding gastro-intestinal symptoms, the patient suddenly felt nauseated and dizzy, vomited a large amount of bloody material, and passed a tarry stool. The episode recurred the following day. Subsequently the patient was hospitalized and given three transfusions for anemia. Menstrual bleeding has never been excessive, and there has been no bleeding from the gums or into the joints.

The patient appeared in good general health and the physical examination was unremarkable except for the presence of a few small ecchymoses on the arms and thighs. There were no petechiae or telangiectases. The mucous membranes appeared normal. There was

no lymphadenopathy and the liver and spleen could not be felt. The joints appeared normal. The blood pressure was normal. There was no anemia, and the volume of packed red cells was 42.0 per cent.

Whole blood clotting time on two determinations was 23 and 34 minutes. One-stage prothrombin time was 19 seconds on two occasions. Platelet counts were 188,000 and 176,000 per cmm. Test of capillary resistance was normal. Whole blood clot retraction test was normal (84 per cent), and the clot retraction test performed on a plasma system gave normal results. The bleeding time was prolonged to 17 minutes. Under the corneal microscope the conjunctival vessels showed a rather marked increase in tortuosity and there were a number of aneurysmal dilatations of the capillaries. After 0.5 per cent pontocaine was applied locally, the conjunctiva was traumatized with a hypodermic needle. This procedure was followed by a rather large subconjunctival hematoma, and the bleeding was such as to obscure all vascular details at the site of the injury. The initial results of the one-stage prothrombin consumption test were equivocal. For that reason the test was repeated on a number of occasions. On three of these occasions the results were normal and on another three abnormal, with failure to utilize prothrombin. The platelets appeared abnormal on a smear stained with Wright's stain. They were large, varied in size, and were irregular in shape. However, the platelets did not appear as abnormal as those of the patient's sister (Case 4) who had had a splenectomy.

Platelet Function as Measured by Clot Retraction in Certain Other Clinical Disorders

"Idiopathic" Thrombocytopenic Purpura: The hemostatic defect which occurs in patients with thrombocytopenic purpura is presumably the result of the reduced number of platelets rather than qualitative platelet defects. Nevertheless, it seemed of interest to determine whether those platelets remaining were capable of functioning normally. Blood was obtained from a patient with "idiopathic" thrombocytopenic purpura (E. D., JHH #550911). A platelet count on this blood was 64,000 per cmm. Using acid citrate dextrose (ACD) solution as an anticoagulant, the platelets were concentrated to a level of 123,000 per cmm. of plasma by centrifugation. This relatively platelet-rich plasma was diluted with platelet-free plasma from the same patient. Very good clot retraction occurred at a platelet concentration of 21,000 per cmm. indicating that the platelets were functioning normally with regard to clot retraction.

Hemorrhagic Disorder Associated with Thrombocytosis: We have encountered a number of patients with a hemorrhagic disorder characterized by purpuric bleeding, associated with an excessively high platelet count and often with a prolonged bleeding time. This situation has been encountered most often in polycythemia vera, chronic myeloid leukemia, and the myeloid metaplasia

syndrome. It seemed possible that the platelets, although present in excessive numbers, were functionally abnormal.

E. H. (JHH #610559), a 40 year old white female with chronic myeloid leukemia of about one year's duration had a persistent bruising tendency. Her bleeding time exceeded 4 hours, the platelet count was 978,000 per cmm. The whole blood clotting time, prothrombin time, and one-stage prothrombin

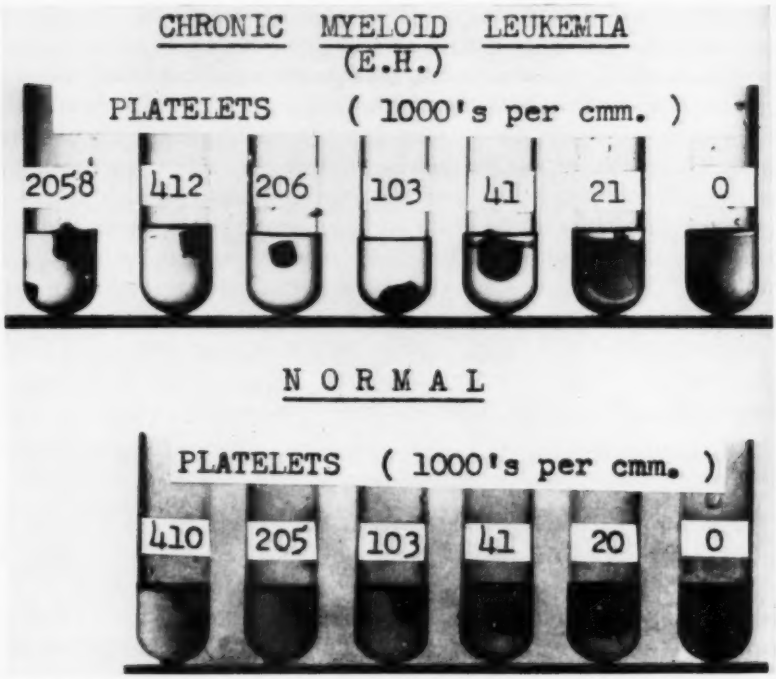

FIG. 6. Clot retraction studies on a patient (E. H.) with chronic myeloid leukemia. Plasmas with varying platelet levels were prepared as in Figure 1. Clot retraction in this case was not significantly different from the normal control.

consumption tests were normal. Clot retraction was studied in a plasma system. The results are shown in Figure 6. It is apparent that clot retraction was normal, even at a platelet concentration of 20,000 per cmm. indicating that the platelets were functioning normally with regard to clot retraction.

M. W. (JHH #141989), a 63 year old colored female has been under observation for 5 years because of a hemorrhagic disorder manifested by excessive bleeding following dental extractions or minor lacerations. The spleen was not palpable when she was first seen but has gradually increased in size so that

it now extends 3 cm. below the costal margin. A moderate normocytic anemia has shown little tendency to progress. The white blood cell count has ranged between 8,000 and 11,000 per cmm., with a normal differential count except that an occasional nucleated red blood cell and a rare immature white blood cell have been seen on the smears. Sternal bone marrow was cellular and showed an increase in the number of megakaryocytes. The platelet count has been enormously increased, averaging about 1,400,000 per cmm. The bleeding time was indefinitely prolonged, and the bleeding had to be controlled by prolonged pressure on the ear lobe. The test of capillary resistance was normal. The whole blood clotting time and one-stage prothrombin time were normal. Study of clot retraction using a plasma system showed good clot retraction even at a platelet concentration of 20,000 per cmm. It seems likely that the patient has one of the myelo-proliferative disorders, possibly the myeloid metaplasia syndrome.

DISCUSSION

A technique for the semi-quantitative measurement of clot retraction employing a plasma system has been applied to the study of patients with certain disease states. Of particular interest was a group of patients with a hemorrhagic disorder associated with qualitative platelet defects—the so-called thrombocytopathic purpuras. Many cases have been reported in which abnormal bleeding has been attributed to qualitative platelet abnormalities. In some of these cases, platelets have been described as morphologically abnormal, in some the clot-accelerating activity of the platelets was thought to be reduced, and in a few clot retraction was said to be impaired. Budtz-Olsen (7) recently has reviewed the reports of cases of so-called "thrombasthenia" in which clot retraction was said to be impaired. He noted that previous reports of defective clot retraction were often based on faulty testing procedures rather than on faulty platelets. He concluded that no case had been reported in which defective clot retraction could be attributed to qualitative platelet defects.

The present studies demonstrate that cases may occur in which a hemorrhagic disorder is associated with the presence of abnormal platelets. Such platelets, though normal in number, may be abnormal to the extent that they are totally incapable of producing clot retraction. It is a matter of considerable importance that in one patient studied (Case 1), defective clot retraction was the only platelet abnormality which was demonstrated. In this case the platelets were morphologically normal and had normal clot-accelerating activity. While it is impossible to prove that there was no other subtle defect in this patient's platelets, the observations suggest that clot retraction function is necessary for the maintenance of normal hemostasis. In another patient (Case 2) whose clot retraction was impaired but not completely absent and whose platelets were morphologically abnormal, there was an associated hemor-

rhagic disorder. Cases of this type certainly lead one to question the suggestion of some investigators (7, 8, 9) that clot retraction is of no physiological significance in the maintenance of normal hemostasis.

That a hemorrhagic disorder may occur in association with a defect in the clot-accelerating activity of the platelets also is apparent as demonstrated in three of the cases herein reported (Cases 3, 4, 5). In these 3 cases, clot retraction function of the platelets was normal although morphologic abnormalities were noted.

In patients with obscure abnormal bleeding and a normal platelet count, one must evaluate platelet function both from the standpoint of clot retraction *and* from the standpoint of clot accelerating activity. Evaluation of platelet function by either of these procedures alone is insufficient to conclude that platelet function is normal. The possibility remains that there may be other independent functions of platelets which are of clinical significance such as vasoconstrictor enhancing activity (10).

Studies on a patient with "idiopathic" thrombocytopenic purpura confirmed the general belief that the clot retraction defect in this disorder is attributable solely to the quantitative reduction in the number of platelets and that those platelets remaining appear to function normally.

A hemorrhagic disorder of unusual interest is that which occasionally occurs in patients with excessively high platelet counts. Some of these patients have been recognized as having chronic myeloid leukemia, polycythemia vera, and the myeloid metaplasia syndrome, while in others the cause of the thrombocytosis was not discovered. The abnormal bleeding tends to be purpuric in character and is usually associated with a prolongation of the bleeding time. Thus the hemorrhagic disorder simulates that seen in severe thrombocytopenia. It seemed possible that the platelets in these individuals were qualitatively defective. For this reason, such patients were studied. No evidence of platelet dysfunction could be demonstrated even in patients in whom the platelets appeared morphologically abnormal, and the cause of the bleeding remains obscure.

In a number of clinical situations, striking abnormalities of platelet morphology may occur. Giant and bizarre platelets frequently are seen in patients following splenectomy, and abnormal platelet forms often are encountered in leukemia. Most remarkable platelet abnormalities may be seen in the myeloid metaplasia syndrome. In some patients with this disorder occasional platelets are as large as red blood cells and fragments of megakaryocytes may be seen in the circulating blood. In none of these conditions in which platelets are morphologically abnormal was there evidence of platelet dysfunction. Even in the so-called myeloproliferative disorders there was no correlation between the degree of morphologic platelet abnormality and the tendency to abnormal bleeding.

SUMMARY

Five instances of a hemorrhagic diathesis associated with a normal number of platelets but with qualitative defects of the platelets (thrombocytopathic purpura) have been reported. In two of these patients, there was a defect in the clot retraction function of the platelets and clot-accelerating activity was normal. In three patients, there was a qualitative defect in the clot-accelerating activity of the platelets and clot retraction function was normal. The clot retraction defect seen in "idiopathic" thrombocytopenic purpura was correlated with the quantitative reduction in the number of platelets, and no evidence was found for an associated qualitative platelet defect. The hemorrhagic diathesis occasionally occurring in patients with thrombocytosis was studied, and no defect of platelet function could be found. The cause of this hemorrhagic diathesis remains obscure. Abnormalities of platelet morphology may be seen in a variety of clinical situations. However, there was no definite correlation between the degree of morphologic platelet abnormality and the presence or absence of a tendency to abnormal bleeding. In patients with abnormal bleeding, the need for evaluating platelet function both from the standpoint of clot retraction function *and* from the standpoint of clot-accelerating activity is stressed since it appears that both of these functions of platelets may be necessary for the maintenance of normal hemostasis.

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HISTOCHEMICAL STUDIES ON CARTILAGE AND BONE

III. Osteogenesis Imperfecta

RICHARD H. FOLLIS, JR.*

Department of Pathology, the Johns Hopkins University School of Medicine

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Recently, evidence was presented (1) to warrant that the osteogenesis imperfecta syndrome be designated an example of "a connective tissue diathesis". A common defect was demonstrated in the bones, cornea, sclera, and corium. In all these structurally related tissues, an abnormality in the development and maturation of collagen was present. In the bones a peculiar basophilic, periodic acid-Schiff positive material was found in place of osteoid; in the other tissues only argyrophylic reticulum fibers were demonstrable. Since the publication of this report (1) we have been fortunate to encounter another case of osteogenesis imperfecta at autopsy and were able to preserve certain skeletal tissues for detailed examination.

CASE REPORT

A colored male infant, weighing 1180 grams, was delivered of a normal mother. The only complication was premature rupture of the membranes. Duration of pregnancy was estimated as 32 weeks. Parents were normal as were 3 siblings who were specifically examined after the autopsy of the present case. Clinical (Fig. 1) and x-ray examination (Figs. 2 and 3) lead to the diagnosis of osteogenesis imperfecta. The child did fairly well for 11 days and then died suddenly for no apparent reason.

Autopsy (23978). There was symmetrical shortening of the extremities, more prominent in the lower than the upper. The distal portions, particularly the feet, tended to taper off in characteristic fashion so as to appear micromelic. There was bending, particularly of the legs. The proportions of the head and body were normal. The remainder of the examination revealed little else save the skeletal changes to be described below. The liver weighed 56 grams; the spleen weighed 5.5 grams.

Tissues were removed as rapidly as possible and placed in 80% neutral ethyl alcohol, acetone and 10% neutral formalin. Blocks were embedded in paraffin or nitrocellulose with or without treatment with 5% nitric acid. Special stains were carried out as described elsewhere (2, 3).

* Present address: University of Utah College of Medicine, Salt Lake County General Hospital, 2033 S. State Street, Salt Lake City 15, Utah.

Microscopic examination

Cartilage: Specimens of costal cartilage and of epiphyseal cartilages of the long bones all showed a similar normal pattern. The sizes and shapes of the cells as they increased in stages of maturation were entirely normal. Scharlach R stains of frozen sections showed the usual cytoplasmic distribution of *neutral fat* (Fig. 7); controlled (with saliva) *glycogen* stains (PAS) showed red deposits in the cytoplasm of the hypertrophic cells (Fig. 6). Preparations fixed in acetone and incubated in the presence of glycerol phosphate exhibited *phosphatase* activity as compared with unincubated controls (Figs. 8 and 9). The intercellular cartilage matrix was normally *metachromatic* and showed normal staining reactions with the periodic acid-Schiff technique. Study of the *anisotrophic properties* of the cartilage matrix with polarized light revealed a normal pattern. Finally, staining of undecalcified sections with silver nitrate (von Kossa) revealed the presence of adequate *inorganic material* in the cartilage matrix between hypertrophic cells. This same picture is seen in control sections for the demonstration of phosphatase activity (Fig. 8).

Invasion of the regularly arranged cartilage cell columns by capillaries was normal. Therefore, from what has been described up to now, growth and maturation of cartilage cells and deposition of inorganic materials in cartilage matrix showed no deviation from normal.

Metaphysis: In the upper or epiphyseal metaphysis all similarity to normal growth sequences ceased (Figs. 4, 5, 12, 13). There was a conspicuous "lattice" of calcified cartilaginous matrix, which was completely bare of osteoid or bone. In addition, there was an absence of typical plump osteoblasts such as one might expect normally in this area. Only spindle-shaped cells were seen. The reduction in numbers and activities of osteoblasts was exemplified by the absence of bone matrix.

As a result of the absence of bone to act as a strengthening agent to encase the spicules of calcified cartilaginous matrix, some of the latter tended to fracture and fragment. Such fractures, however, did not appear to be associated with the appearance of another abnormal finding which was unique. A little beneath the cartilage shaft junction, a bluish-staining (with hematoxylin) material was found surrounding the spicules of calcified cartilaginous matrix. This material had a granular unhomogenous appearance. It seemed to be the result of cellular activity, since cells resembling osteocytes were embedded in it. Basophilia of newly deposited normal unimpregnated (or even inorganically impregnated) matrix is not encountered in normal bone. Further evidence for the pathologic nature of this material was its staining reaction with the PAS reagents. An intense red color was produced, more brilliant than is exhibited by normal cartilage matrix or bone matrix (osteoid) (2). The material stained

metachromatically with toluidine blue in decalcified sections, but not as intensely so as does cartilage matrix. Stains for iron with the Prussian blue reagents (potassium ferrocyanide and hydrochloric acid) were negative.

Another feature which was abnormal was the presence of an eosinophilic material in the clefts between and about fractures of the calcified cartilage matrix. The nature of this subtance is not clear.

The absence of plump, normally appearing osteoblasts in the metaphyseal region has already been mentioned. Cells which were present had elongated spindle shapes and less basophilic cytoplasm than one ordinarily expects in the functioning osteoblast. Phosphatase activity was present in these cells, however.

There were virtually no osteoclasts in the metaphysis, except when fractures of the lattice had occurred (Fig. 22).

Periosteum and Cortex: Defective osteoblastic activity was seen to good advantage in this area. The layer of periosteum contained more cells than normal (Figs. 16 and 17). There was very little cortical bone. What bone was present seemed normal as far as its osteocyte pattern was concerned. There was a great deal less of it, however. The cortex was thus very thin or even lacking. Hence, as might be expected there were many breaks and infractions. Pink staining material similar to that noted above was also present. Phosphatase activity associated with periosteal cells was normal (Figs. 10 and 11). Glycogen was present in the cytoplasm of some osteoblasts.

Diaphysis: As a result of the thin cortex and presence of spicules of calcified cartilage matrix unencased by new bone in the diaphyseal region it is obvious that many spontaneous fractures would have occurred. Such areas showed evidence of new bone formation of a membranous type such as one would expect to find in the usual fracture healing site. One found, therefore, three types of tissue: an inner core of calcified cartilage matrix, a middle zone of basophilic (and PAS positive) material and an outer covering of bone (Figs. 14, 18, 19, 20). The fibrillar nature of this latter was normal (Fig. 21).

Another feature of the diaphysis was the presence of islands of cartilage beneath the periosteum (Fig. 15). Such islands were obviously related to fractures and represent an abortive formation of callus in such areas.

Marrow Elements: Marrow cells, erythrogenic and myelogenic were present but were scanty in areas where fractures had occurred. Therefore, beneath the epiphyseal or costal cartilages in the area where fractures of the calcified cartilage matrix were occurring there were few marrow cells. The marrow framework consisted of a loose cellular and fibrillar material. When fractures were not present marrow cells were plentiful.

Membrane Bone: Sections of the calvarium showed some, though very little, membranous bone formation. It was identical, as one might expect, with the type of structure found in the periosteal regions described above.

DISCUSSION

It is not the purpose of this discussion to review in any detail the many histological studies (4, 5, 6, 7, 8, 9, 10, 11, 12) which have been carried out on cases of osteogenesis imperfecta since the first detailed report by Stilling (4) in 1889. Certain aspects of the skeletal lesions deserve emphasis, however, in relation to the observations presented above.

In the first place, it is clear enough from a study of our own material and from that of others that growth and differentiation of cartilage is normal. In other words, the development of the cartilagenous skeletal anlage from the primitive mesoblastic cells which give rise to it is entirely normal. So too, growth and maturation of the various cartilages, their central invasion by blood vessels and the formation of a marrow cavity so that epiphyses are developed, all conform to the normal pattern. The histochemical studies on cartilage which we have detailed above would further point to the normalcy of cartilage growth and development. Fat is present; the hypertrophic cells contain glycogen. The intercellular matrix has abundant phosphatase activity. So, too, the calcification mechanism in the cartilage would appear normal since inorganic materials are present in adequate amounts in the provisional zone of calcification.

However, it is when one comes to examine the osteoblastic sequences, endosteal and periosteal, that a serious defect appears. This abnormality has been commented upon in numerous autopsy studies of osteogenesis imperfecta. As far as endosteal function is concerned, osteoid, the organic matrix of bone fails to appear on the scaffolding of calcified cartilagenous matrix. Instead a peculiar basophilic, PAS positive material makes its appearance. The basophilia has been commented upon by others (5, 7, 9). The peculiar staining reaction of this material with the periodic acid-leucofucsin technique was first described in a previous report (1). Exactly what this material is cannot be determined. It is of interest that it displays the same staining properties as reticulum and like the latter is argyrophilic. The only material in bone which even stains so deeply with PAS are so-called "cement lines". Neither collagen from the corium or osteoid stain deeply with the PAS technique nor are they argyrophilic. If reticulum is an immature form of collagen or a form in which perhaps polysaccharides such as chondroitin sulfuric acid or hyaluronic acid are bound so that they might stain with the PAS reagents, then a similar situation might be present in the matrix (if such it can be called) in osteogenesis imperfecta. Hence this basophilic material might be termed an "immature form of bone matrix" (1), a highly speculative assumption, however.

Osteogenesis imperfecta must be regarded as a disease resulting from derangement of osteoblastic activity. Growth of cartilage appears normal. There is thus a complete dissociation of chondroblastic and osteoblastic activities. Such

a separation of these activities is, of course, not uncommon. One is familiar with the formation of zones of increased density and transverse lines in the metaphyses associated with disturbances in growth activity of cartilage cells and osteoblasts (13). Perhaps the best example of complete cessation of osteoblastic activity is found in scurvy as one sees it naturally occurring in the infant (14) or in the experimental animal (3). The histological picture in scurvy is not unlike that seen in osteogenesis imperfecta. In fact, one could pick almost identical microscopic fields in either. Congenital scurvy has, of course, been described (15) but much more bone formation occurs *in utero* than in cases of osteogenesis imperfecta. Another interesting bone disturbance, in which osteoblastic activity is impaired while growth of the cartilage cells goes on, is found in copper deficiency in dogs (16). The dissociation of chondrogenic and osteoblastic activities in such a circumstance only occurs after animals have been on a copper deficient regimen for some time.

There is no evidence that osteogenesis imperfecta is due in any way to a disturbance in calcium or phosphorus metabolism (17). Metabolic studies have not shown any different pattern save a negative balance, which might be expected if no bone matrix were present for calcium and phosphorus to be deposited. Elevated alkaline phosphatase activity of the serum has been noted; this, of course, can easily be explained by fracture healing.

The osteogenesis imperfecta syndrome is truly an enigma of the osteoblast (and fibroblast, too). Why cannot bone matrix be formed in normal fashion until fractures occur? If one knew the answer to this one might know a great deal more about the formation of collagen specifically and of all protein in general.

SUMMARY

Histochemical observations have been presented in a case of osteogenesis imperfecta. Such studies verify the normalcy of cartilage growth and development in this syndrome, and indicate, though do not explain, the primary defect which is a derangement in osteoid formation, doubtless due to some defect in the osteoblast.

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PLATE I

FIG. 1. External appearance of infant. Note normal sized head and trunk. Arms and, in particular, the legs are shortened and exhibit typical tapering. Legs show more pronounced bowing than arms.

FIGS. 2 AND 3. Roentgenograms (postmortem) of upper and lower extremities. Note numerous fractures with evidence of repair. Bowing of tibia is especially prominent.

FIG. 4. Low power photomicrograph of costochondral junction. Cartilage is normal. Note disorganization of underlying cortex and trabeculae. The shaft bulges out and it is clear that there has been some compression of the cortex. This is brought out in the higher power view of *Figure 5*. Note large number of osteoblast-like cells in marrow space.

FIG. 5. See above.

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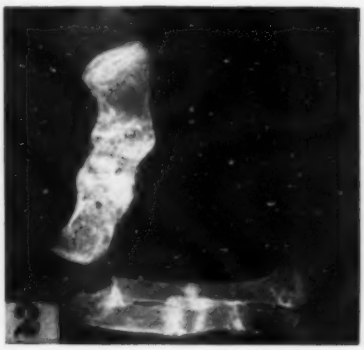

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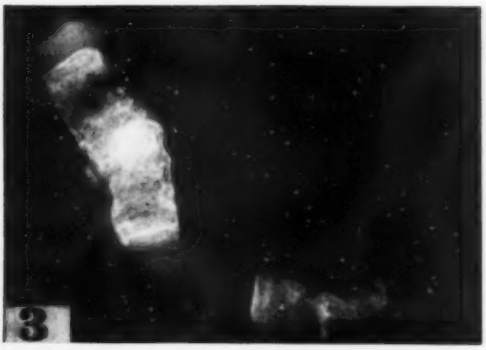

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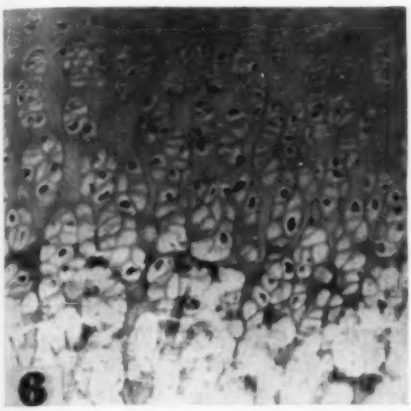
PLATE II

FIG. 6. Glycogen stain (periodic acid-Schiff technique) of alcohol fixed, undecalcified section to show presence of dark staining material (actually red) in cytoplasm. Nuclei stain very lightly in the preparation. Material is removed by incubation with saliva.

FIG. 7. Fat stain (Sudan IV) of frozen section of costal cartilage to show presence of neutral fat globules in undifferentiated cartilage cells.

FIGS. 8 AND 9. Comparable sections of alcohol fixed, undecalcified costochondral junctions. No. 9 was incubated with glycerol phosphate in the presence of excess calcium salt. Both sections then were stained to bring out calcium phosphate deposition. Note normal activity in *Figure 9*. *Figure 8* brings out normal impregnation of matrix in osteogenesis imperfecta.

FIGS. 10 AND 11. Phosphatase activity. Comparable sections of skull bone. *No. 11* was incubated with glycerol phosphate and treated in usual way. Note normal enzyme activity, especially in periosteum.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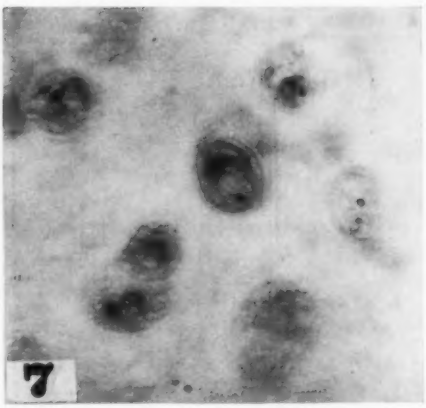
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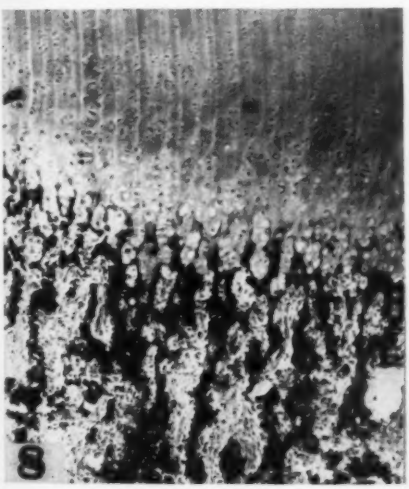
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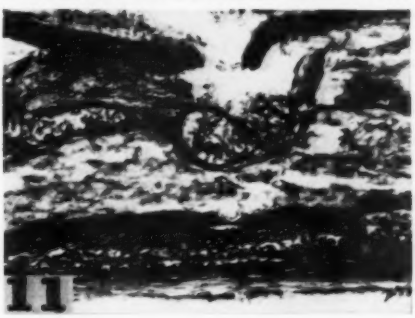
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PLATE III

FIG. 12. Cartilage shaft junction of upper end of tibia. Note normal columnar arrangement of cartilage cells. Intercellular matrix is impregnated with inorganic materials but contains relatively little bone on it. Note disorganization below.

FIG. 13. Higher power of region similar to that shown in Figure 12. Note dark staining material deposited on cartilage matrix. This is periodic acid-Schiff positive. This material is most prominent in areas where fractures of calcified matrix have taken place. Note cellularity of marrow space; cells present resemble osteoblasts.

FIG. 14. Higher power to show fractures and disorganization of spicules of calcified matrix encased in dark staining material.

FIG. 15. Mass of cartilage in cortex of lumens in area of transverse fracture. These masses of cartilage are interpreted as being a part of the callus which forms when fractures occur

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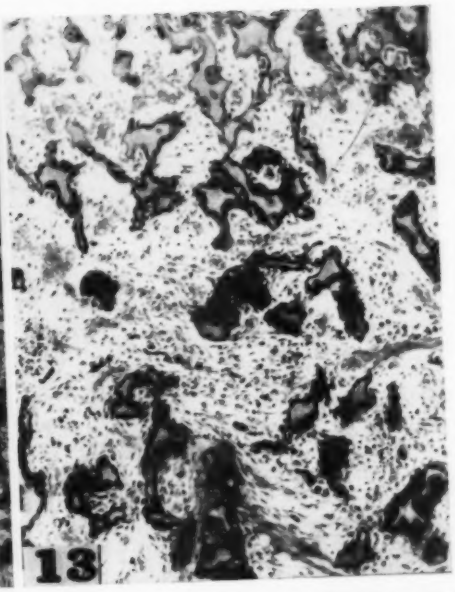
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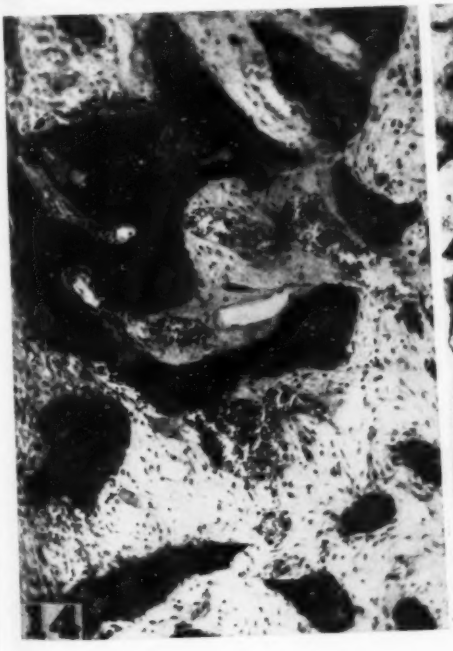
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PLATE IV

FIGS. 16 AND 17. Sections of periosteum from a normal (16) and from the present case (17) (some magnification). Note increase in thickness of layer of differentiated periosteal cells. Nuclei are more vesicular.

FIG. 18. Higher power to show a spicule of matrix (left) surrounded by a thin layer of dark staining material. Note osteoclasts and osteoblasts. A small trabecula of bone seems to have been formed by the latter.

FIG. 19. New bone formation going on in normal fashion in vicinity of a trabecula.

FIG. 20. Silver stain (Gomori) of fractured cartilage matrix to show dark staining material about fragments. This has no fibrillar properties with this stain.

FIG. 21. In contrast to *Figure 20* the new bone matrix which forms has a definite fibrillar structure (silver stain).

FIG. 22. A mass of calcified matrix surrounded by typical dark staining material. Note prominent osteoclasts along surface.

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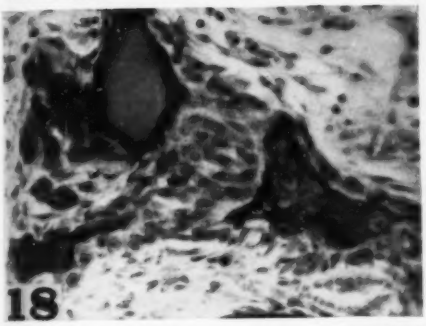
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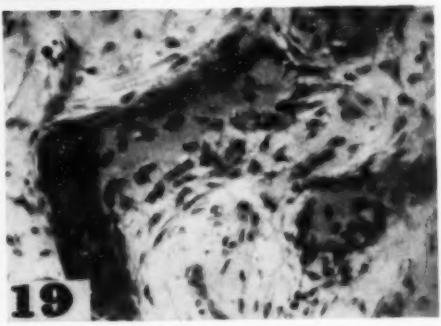
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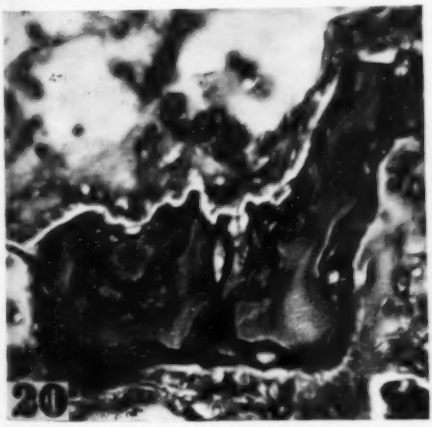
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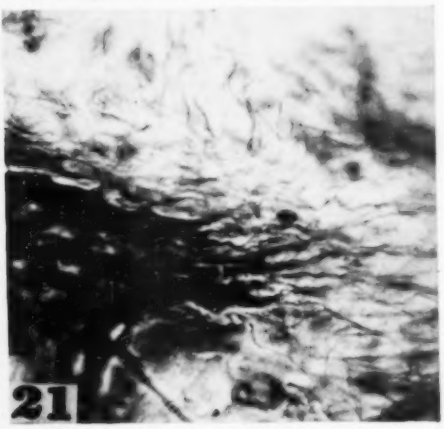
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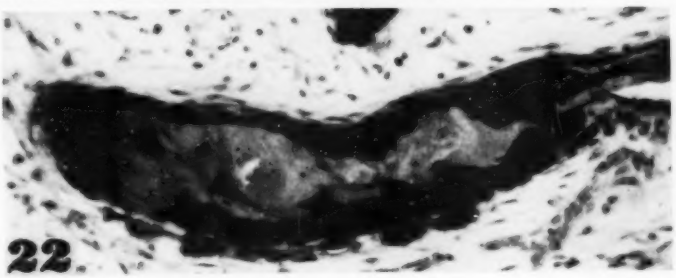
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THE EFFECT OF BLOOD VESSEL PULSATIONS ON LYMPH PRESSURE IN LARGE LYMPHATICS

ROSCOE C. WEBB, JR., AND T. E. STARZL

Department of Surgery, the Johns Hopkins University School of Medicine and Hospital

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Rhythmic alterations in lymph pressure at the same rate as the heart beat have long been believed on circumstantial grounds to be an important factor in the propagation of lymph. It was not until 1939, however, that the presence of such lymphatic pulsations was demonstrated by Cressman and Blalock (1), who suggested that the impulses were transmitted from contiguous arteries. By measuring lymph output from local areas to which extrinsic pulses could be imparted or withdrawn at will, Parsons and McMaster (2, 3) have shown that lymph formation and drainage are remarkably facilitated in regions subjected to transmitted arterial pulsations.

Because it had never been proved that rhythmic intralymphatic pressure changes are actually due to transmitted arterial pulsations, the present study of the origin and fate of intralymphatic pulsations was undertaken.

METHODS

Thirty-five large mongrel dogs were used, and technically satisfactory conditions were realized in 20 experiments. The dogs were given condensed milk and 30 minutes later were anesthetized with 30 mgm. per Kg. of nembutal given intravenously. In most of the experiments the chest was opened and a positive pressure respirator used thereafter. Umbilical tapes for controlled occlusions were placed about the aorta, the two vessels arising from the aortic arch, and the left innominate vein through a thoracotomy in the left 3rd interspace. The abdominal aorta was isolated through a separate incision. Arterial, venous and lymphatic vessels were cannulated with polyethylene catheters or blunt 20 gauge steel needles filled with heparin. Inductance type transducers were used to measure arterial and venous pressures; lymphatic pressures were detected with a capacitance manometer. All pressures were recorded simultaneously on a multichannel direct writing oscillograph. The frequency response of the measuring and recording systems employed was in excess of 50 cycles per second when tested by recording pressure within a balloon during explosion.

Two sites were chosen for study: the lower portion of the thoracic duct, which was exposed at the level of the 11th and 12th thoracic vertebrae through a thoracotomy in the 11th left interspace, and the terminal portion of the thoracic duct, which was exposed through a left supraclavicular incision. In each location pressures were obtained either by cannulating a tributary so that the main lymphatic channel was unobstructed, or by directly cannulating the thoracic duct in which case the central lymph flow was blocked. In this paper the former method is referred to as *side pressure measurement*, and the latter as *end pressure measurement*.

Aided by a grant from the United States Public Health Service, National Institutes of Health, Bethesda, Maryland.

In order to measure end pressures the lymphatic channel was mobilized over a distance of 8 to 10 mm. and lifted carefully so that the recording system and lymph vessel would be completely free of contact with any other structure, insuring that any pressure alterations recorded were discretely intralymphatic. Side pressures were obtained by placing the polyethylene tube or blunt steel needle through a tributary until its tip reached the main lymphatic channel. In this situation all other vessels in the immediate vicinity which might impart movement artefact to the recording system were retracted or removed.

RESULTS

Recording from the thoracic duct just above the diaphragm. Mean side pressures ranged from 35 to 55 mm. water. After several minutes of obstruction to flow,

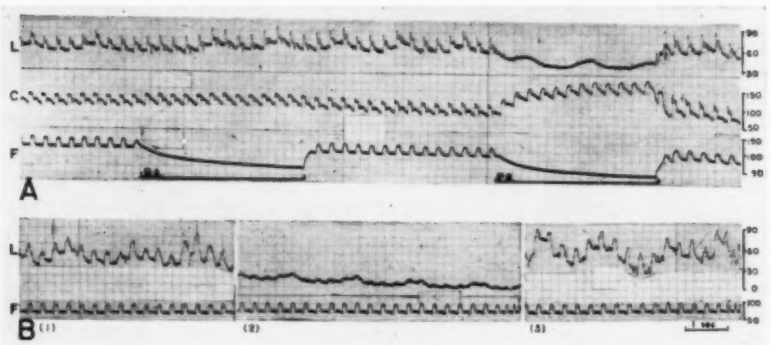

FIG. 1A. Side pressure recording from thoracic duct at the level of T_{12} showing lymphatic pulsations (L) and how these were unaffected by aortic occlusion below the renals (DA) and eliminated by occlusion at the first intercostal level (PA). Concurrent right common carotid (C) and left femoral (F) arterial tracings are shown.

B. Similar record (1) in which a ligature tightened in position to prevent lymph from reaching the tip of the recording catheter obliterated both pressure and pulsations (2), with restoration of the original pattern after ligature was loosened (3). Lymph pressure calibrations are in mm. saline, and arterial pressures in mm. mercury.

end pressures were as high as 350 mm. water, but by intermittent release of the lymph between recordings the pressures were generally maintained at less than 100 mm. water. There were only two types of demonstrable variations from the mean level. The first type consisted of respiratory alterations (Figs. 1–3), which could be kept at a minimum by maintaining the positive pressure respirator at a low exchange. The second type of pressure variation consisted of rapid lymphatic pulsations in phase with the heart beat, which were essentially the same irrespective of whether side or end pressure furnished the background. The pulse pressure of these rapid lymphatic cycles was surprisingly great, usually being 20 to 30 mm. water and amounting to a significant fraction of the peak lymph pressure. Thus typical pressures in millimeters of water could be tabulated as 70 systolic and 40 diastolic, or 55 systolic and 35 diastolic.

The pattern of the lymphatic pulse tracing was variable from animal to animal and at different times in the same animal, sometimes being very sharp with complex form (Fig. 4A), and at other times being a simple wave (Fig. 1B). In all instances, however, the chief (always the first) component had a fixed time relationship to the arterial pulse (Fig. 4A), starting after the carotid and before the femoral pulsations in the position one would predict for adjacent aortic pulsations.

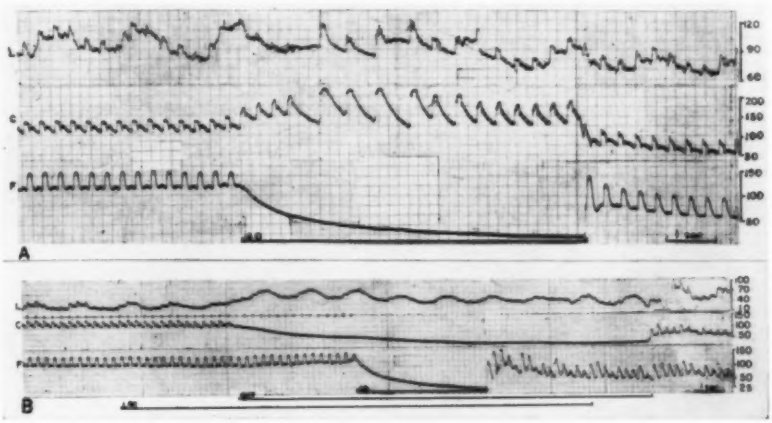

FIG. 2. Side pressure recordings showing effect upon pulsations in the cervical thoracic duct (L) of: A. Occlusion of the aorta at the first intercostal (AO), causing a carotid hypertension with concurrent augmentation of the lymphatic pulsations. B. Serial occlusion and release of the left subclavian (LSC) and brachiocephalic (BCT) arteries, and the thoracic aorta at the first intercostal (AO).

Right common carotid (C) and left femoral (F) arterial pressures are included. The vertical indicator dashes beneath the lymph recording in B show the time of onset of femoral pulses. Lymph and venous pressure are in mm. saline, and arterial pressures are in mm. mercury.

Occlusion of the aorta distal to the site of the lymphatic catheter had little or no effect on the lymphatic pulsations (Fig. 1A), whether the occlusion was at the level of the superior mesenteric artery, renal arteries, or at the bifurcation. Similarly, occlusion of the ascending lymphatic channels at any point 2 or 3 cm. or more below the level of the cannulation did not appreciably alter the pulsations although the mean lymphatic pressure generally fell.

On the other hand, occlusion of the aorta proximal to the lymphatic cannula always completely obliterated the intrinsic lymphatic pulsations (Fig. 1A), usually with an attendant fall in mean lymphatic pressure. Pulsations promptly reappeared upon release of the aorta.

That the pulsations recorded were representative of pressure events in the

lymph rather than of rhythmic catheter movement or other artefact, was evident from several considerations. With either end or side pressure measurement, when the tip of the lymph filled catheter was trapped by a ligature *in situ* so lymph could not reach it (Fig. 1B), the pressure fell and pulsations ceased, to return agair when intralymphatic contents were brought back into

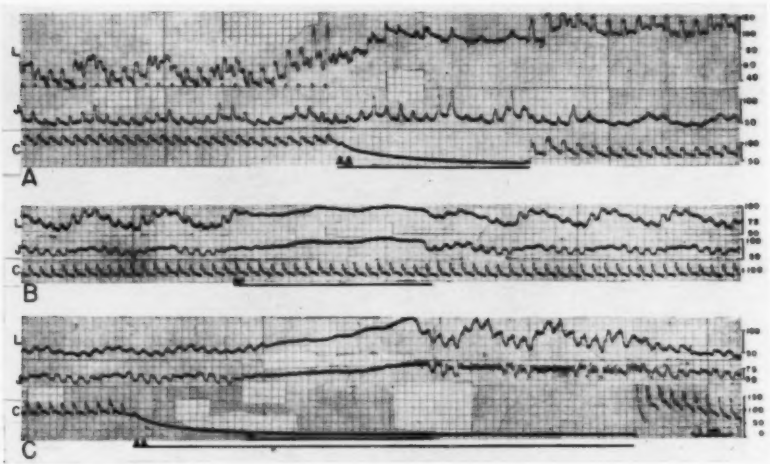

FIG. 3. Side recordings from the cervical thoracic duct (L) showing in each case a transmitted venous contribution to the lymphatic pulsations. Concurrent pressures were taken from the internal jugular vein (J) and left common carotid artery (C). Lymph and venous pressure calibrations are in mm. saline and arterial pressures in mm. mercury.

A. Effect of ascending aortic occlusion (AA), showing elimination of the arterial component of the lymph pulsations. Vertical indicator dashes in lymph channel mark onset of left common carotid pulses.

B. Different animal showing disappearance of pulse and elevation of mean intralymphatic pressure upon occlusion of innominate vein (IV).

C. Immediately after record shown in B the thoracic duct was ligated at its entry into the left subclavian vein. Pulsations persisted, were not affected by ascending aortic occlusion (AA), but were abolished by innominate vein occlusion (IV).

contact with the recording system. Furthermore, it was demonstrated grossly by several techniques that the contents of lymphatic vessels pulsated with considerable sharpness and vigor. When a catheter was placed in the thoracic duct, the lymph could clearly be seen to pulsate as it rose in the tube; or if a small opening was made in the vessel wall, the escaping lymph was seen to issue in a pulsatile manner. Moreover, if either a small air bubble or a tiny amount of ink was injected into the duct, it slowly passed rostrally, moving about with each aortic pulsation, pausing transiently at the valves, and finally passing out of sight into the neck.

Recording from the cervical thoracic duct. Mean side pressures ranged from 25 to 100 mm. water, and mean end pressures were maintained at approximately 100 mm. water by intermittent lymph letting to relieve the obstruction. Intralymphatic pulsations, similar to those described above, were evident. The lymph pulsated either in synchrony with adjacent arteries (Fig. 2) or in

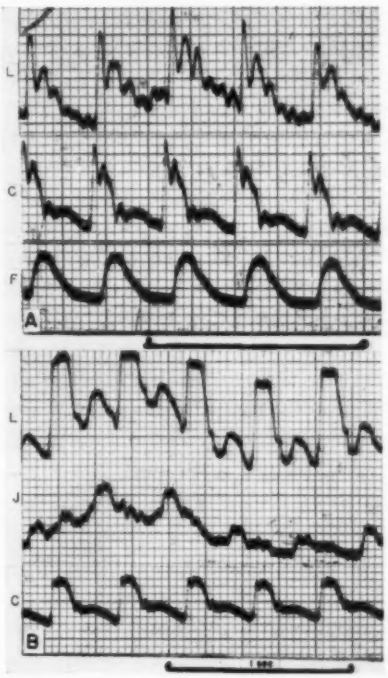

FIG. 4. Lymphatic pulsations in magnified detail showing time relationship to pulsations of adjacent vessels.

A. Side pressure recording from thoracic duct at the T-12 level. The record is an enlarged segment of that illustrated in Fig. 1A, and has the same legend.

B. Similar enlarged segment of record obtained from the cervical thoracic duct. The record is a magnified portion of that shown in Fig. 3A, and has the same legend.

phase with the pulsations of the neck veins (Fig. 3B, C). Often pressure changes were coordinated with both arterial and venous pulses, resulting in an irregular double lymphatic pulse for every cardiac cycle (Figs. 3A, 4B).

The transmitted arterial component of the lymphatic pressure waves was synchronous with the carotid (Figs. 2, 3A, 4B) or subclavian pulse. Accordingly, if the blood or pulse pressure in these vessels was altered, dramatic changes occurred in the lymphatic pulsations. When the third portion of the

aortic arch was occluded (Fig. 2A) carotid hypertension and widening of the arterial pulse pressure occurred immediately, accompanied by augmentation of the lymphatic pulsations. Conversely, if the arteries of the head and neck were serially blocked a decrement or a fall-out of lymph pulsations was demonstrable. Such an instance is shown (Fig. 2B) in which left subclavian occlusion caused a reduction in size of lymph pulsations, followed by virtual disappearance of pulsations when the brachiocephalic artery was occluded. Minute pulsations which remained could then be abolished by occlusion of the third portion of the aortic arch. More simply, occlusion of the ascending aorta also eliminated the transmitted arterial element of the lymphatic pulse, leaving, in those instances where it had been present before (Fig. 3A), a lymphatic pressure wave related only to venous pulsations.

Alterations of lymphatic pressure that occurred in phase with the jugular or subclavian venous pulsations could be recorded with either end or side pressure methods, suggesting at the outset that they were transmitted by contiguity rather than by retrograde flow from the subclavian vein into the thoracic duct. This component of the lymphatic pulse was not significantly affected by occlusion of the ascending aorta (Fig. 3C) but was eliminated by occlusion of the innominate vein (Fig. 3 B, C) or the superior vena cava. Ligation of the thoracic duct at its entry into the subclavian vein, which broke intraluminal contact between the two systems, failed to eliminate this type of lymphatic pulse (Fig. 3 B, C)—emphasizing that any venous component of the lymphatic pulses is chiefly activated by adjacent pulsating veins rather than by venous reflux into the duct.

DISCUSSION

With recording techniques and by direct observation, it is evident that movements from the great arterial trunks are imparted to the larger collecting lymphatics. These transmitted pressure changes apparently keep the lymph in continual pulsatile motion which, because of the high degree of valve competence (1), is of necessity oriented toward the venous drainage sites. In the neck, where the thoracic duct is in close relation to large pulsating veins, venous pulsations are similarly transmitted to the lymph, a process which is probably peculiar to a few anatomic areas such as the subclavian triangle and of limited functional significance.

The type of experimentation performed on the lower thoracic duct yielded valuable information on the fate of the transmitted intralymphatic pressure alterations. Occlusion of the aorta just proximal to the level of pressure measurement routinely obliterated the lymphatic pulses at distal levels, indicating that retrograde lymphatic conduction of these transmitted effects does not occur. Similarly, blockage of ascending lymph channels or elimination of

distal aortic pulsations did not appreciably alter the lymphatic pulsations, suggesting that rostral propagation of the lymph pressure changes is likewise for very short distances.

The information presented is directly relevant only to the effect of the great arteries on the larger lymphatic channels. The possible extension of such a concept to the distal vascular and lymphatic beds has been stressed in the skilful experimentations of Parsons and McMasters (2, 3), although at present one can only speculate upon the role of this mechanism in peripheral lymph clearance.

SUMMARY

A study was made in dogs of rhythmic intralymphatic pressure changes in the supradiaphragmatic and cervical thoracic duct. In both areas lymphatic pulses were found to be transmitted from contiguous large arteries, the lymphatic pulse pressure representing a significant fraction of the peak lymphatic pressure. In the neck venous pulsations were also shown to be transmitted to the lymph in a similar manner. These pulsations were shown to be propagated for only a few millimeters. By "tagging" the lymph it could be demonstrated that the pulsations were associated with its proximal flow. The possible functional significance of such a mechanism in general lymph clearance is reemphasized.

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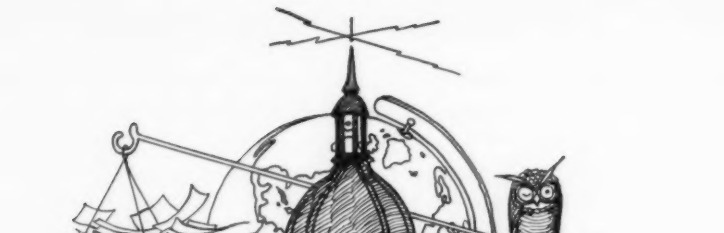

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of the Bulletin)

Children of Divorce. By J. Louise Despert. 282 pp., $3.50. Doubleday & Co., Inc., Garden City, N. Y.

The first reaction on reading this book is astonishment that it is the first of its kind. Written for the one in three husbands and wives who are divorced and on behalf of their approximately 400,000 children in this country it meets an obvious and widespread need.

The book is concerned with how to make the best of a bad job. While making no attempt to minimize the tragedy of divorce, especially in its effects on children, it repeatedly stresses that parents who are concerned about the welfare of their children can do much to help them adjust to their shattered world. It abounds in sound, helpful, concrete advice on how to help children of different ages to cope with almost any conceivable contingency before, during and after divorce.

Certain themes recur. One is that it is feelings, not external acts, which count for the child, that, for example "emotional divorce" of parents who continue to maintain the shell of a common home may do more harm to the children than an open break. Another, equally valid, is the importance of recognizing and dealing with the child's guilt arising partly out of a feeling that his parents' troubles are his fault, partly out of his anger towards one or both for letting him down.

Points are illustrated with moving, sensitively written case histories, some describing the damaging effects of divorce on children, others dealing with those who managed to come through this inevitably harrowing experience successfully. Trenchent criticism of our misguided and chaotic divorce laws is balanced by discussion of hopeful new trends for dealing with divorce as a human rather than a legal problem and making the welfare of the children the primary concern

This book should be helpful to persons who are involved in divorce or are consulted by those who are.

Jerome Frank

Microbiology and Human Progress. By MADELEINE PARKER GRANT. 718 pp., $6.75. Rinehart & Co., New York.

The author, who is professor of Biology and Bacteriology at Sarah Lawrence College, sums up the aims of this somewhat unique book when she says she hopes it will serve as a text-book of biology and that it will emphasize the effects of microorganisms on the life and death of man and give the student insight into the interdependence of science and society. This is a large order, but it seems to have been filled remarkably well. The historical landmarks in microbiology and medicine are presented in a concise and entertaining manner, covering the main steps in our knowledge, and bringing us up to date in each subject. All this is done without resorting to the sensational methods of reporting so frequently employed by those who attempt to translate the progress of this science to the public. A high plane of critical and scientific writing is maintained throughout the rest of the book also.

It is impossible to do justice to a work of this sort in a short review as it covers so many fields, but some of the high points will be mentioned. The observations made on the similarity between genes and viruses are quite pertinent. Here we have two minute living substances that are in some ways self-perpetuating, each capable of directing the metabolic activity of the host cell, and probably both complex protein molecules. Genes are referred to as "possible ultimate units of life" and viruses are sometimes regarded as "states of matter that bridge the gap between the protein molecule of the chemist and the organism of the biologist." Bacteriophages—viruses that feed on bacteria and multiply rapidly in the process—are good examples of these minute organisms that may bridge the gap.

There is a particularly good chapter on heredity with a discussion of variation and mutation in bacteria. The significance of these phenomena is pointed out in relation to studies on resistance of bacteria to antibacterial agents, as well as to a broader understanding of human evolution and genetics.

The major role played by microorganisms in many types of industry and in farming is brought out in another section showing the growing importance of the relatively new field of microbiology. A novel and interesting viewpoint is taken of health, which the author feels should be considered as a dynamic or positive condition rather than just an "absence of disease." A rough estimate of a person's health or his chances against disease can be determined, if we attempt to balance the forces of one against the other. The main features dealing with infections, immunity and therapy are well described as are most of the infectious diseases.

The philosophical viewpoint is stressed repeatedly, being illustrated by comparisons between various aspects of microbic and human life. The author has the ability to make her material accurate and sufficiently inclusive without its being either too long or boring. It is a book that should prove to be instructive not only to the undergraduate student of biology, but also to others farther along in this field.

A. MURRAY FISHER

Adrenal Cortex. Transactions of the Fourth Conference, November 1952. Edited by ELAINE P. RALLI. 165 pp. $4.00. The Josiah Macy, Jr. Foundation, New York.

This small book reports discussions on the physiology of the adrenal cortex by a number of leading investigators assembled at The Josiah Macy, Jr. Conference. The informal nature of the conversations has been preserved so that the numerous questions and arguments bring forth many excellent points and enable the reader to see various viewpoints of controversial issues.

Dr. Ingle discourses on a theory of the permissive, as compared to the regulatory, action

of adrenal cortical hormones. This is illustrated by the results of experiments which determined the effect of adrenal cortical hormones on variations in the degrees of glycosuria produced by different agents in the partially depancreatized rat. The effect of epinephrine in causing a decrease in blood eosinophiles is then discussed and it is the general consensus of opinion that this phenomenon does not represent an index of adrenal cortical activity. It has been shown that no increase in the secretion of adrenal cortical hormones occurs after the administration of epinephrine and that the drug can cause an eosinopenia in the adrenalectomized organism. The fall in eosinophiles seems therefore to be mediated through an extra-adrenal action of epinephrine.

In the second chapter Dr. Selye attempts to explain his theories of the alarm reaction and presents data on the induction of hypertension and nephrosclerosis in animals with the use of salt and DCA. He also mentions that STH (growth hormone) can cause adrenal hypertrophy, and can prevent the adrenal atrophy produced by cortisone. Dr. Li states that growth hormone can be demonstrated in the plasma of patients with active acromegaly, but not in that of growing children. Then follows a debate on why growth hormone fails to induce growth in man, but no definite answer to the question is forthcoming. Dr. Sayers comments on a new assay for ACTH in plasma and shows that there is a high level of ACTH in the plasma of animals subjected to stress. At this point Drs. Selye and Sayers indulge in a lively argument on the merits of their theories of regulation of pituitary-adrenal cortical activity and the other experts find faults with both theories.

The third chapter deals with some recent data presented by Dr. Vogt on the existence of a new mineralocorticoid which has been discovered in adrenal extract and in the adrenal venous blood of dogs and monkeys and which is 20 times as potent as DCA with respect to salt retaining activity. Dr. Pincus expounds on the synthesis of adrenal steroids as demonstrated in experiments in which beef adrenal glands are perfused, and on the degradation of adrenal steroids into 17-ketosteroids. The role of the liver in the latter process is regarded as important.

In the last chapter Dr. Lukens presents some interesting data on the results of complete adrenalectomy in man as a treatment for hypertension. Dr. Thorn and others join in a discussion of the results of such therapy and of methods of maintaining the patients postoperatively with DCA and cortisone.

There are certainly many new salient facts and ideas on adrenal physiology presented in this book which should be most enlightening for the student and research investigator interested in this field. However, the report will not provide much aid to the practicing physician in the diagnosis and treatment of disease.

JOHN G. WISWELL

Die Lungenresektionen. By A. LEZIUS. 143 pp., Ganzleinen DM 49.80. Georg Thieme Verlag, Stuttgart, Germany.

Since the pulmonary segment has achieved surgical maturity, several books have been published on the subject of bronchopulmonary anatomy and the technical aspects of pulmonary resection. This volume follows the previously established pattern. The text is concise and comprehensive in its coverage and the ideas expressed are in keeping with the best standards of thoracic surgery. In stepwise fashion the operative technic is described for each lung, lobe, and segment. By the liberal use of excellent illustrations Professor Lezius has surpassed his predecessors. There is little doubt that this volume will serve as a valuable German language reference work for thoracic surgeons.

To this reviewer it seems a pity that the author did not confine himself to a purely techni-

cal monograph. Instead he has chosen the customary course of flanking this material with brief sections devoted to clinical matters, pulmonary physiology, differential diagnosis, pre- and postoperative care. The coverage of these subjects is so superficial as to detract from the main body of the work. It is also to be regretted that the author did not see fit to include a bibliography.

In the opening section, which is devoted to the history of pulmonary resection, Professor Lezius challenges the priority of Doctor Graham in having performed the first successful pneumonectomy. He cites four successful pneumonectomies performed in 1931 and 1932 by Nissen, Haight, Windsberg and himself. In fact, he even raises the question as to whether Doctor Graham performed a total pneumonectomy since the lung was removed by tourniquet technic presumably leaving residual lung tissue in the region of the hilus. In this contention it would seem that Professor Lezius is, to a large extent, "shadow boxing" in that Doctor Graham claims priority for having performed the first successful one-stage pneumonectomy whereas the previous cases had been multiple stage procedures.

RICHARD F. KIEFFER, JR.

Hormonal and Neurogenic Cardiovascular Disorders. By WILHELM RAAB. 722 pp., $15.00. The Williams and Wilkins Co., Baltimore, Md.

In the first section of his book the author reviews in great detail experimentally observed effects of hormones and neurohormones ("chemical mediators") on the cardiovascular system. The second section is devoted to an analysis of the cardiovascular aspects of endocrine diseases. The last section is a discussion of endocrine and neuro-endocrine factors in cardiovascular diseases. In the last section, in particular, Raab's unconventional views on the importance of certain factors of these categories is discussed *in extenso*. Specifically his main thesis is as follows: The "ubiquitous catecholamines", arising both in the adrenal medulla and at autonomic nerve endings, play a leading role in pathogenesis of cardiovascular disease. Their oxygen-wasting (locally hypoxiating) action, on the myocardium, for instance, is of great importance in the understanding of angina pectoris, "sudden death without morphological substrate", and, to some extent, Raab would have us believe, in most cardiovascular disorders. Raab believes the effect of thyroid hormone is mainly one of potentiation of the catecholamines. Similarly he believes the pressor effect of the mineralocorticoids operates via a potentiation of catecholamines through shifts in intra- and extracellular ion distribution. The excretion of catecholamines by the kidney may represent a source of local toxic action, whereas their retention in renal insufficiency threatens the cardiovascular system in general. Cardiac hypertrophy cannot be explained, alone at least, on mechanistic, teleological grounds. Raab assembles evidence that the prerequisite for myocardial hypertrophy is increased myocardial oxygen consumption, such as is elicited by catecholamines, regardless of the degree of change in work load. He points out that hypertrophy can be produced by growth hormone, thyroid hormone, and adrenal corticoids without definite relation to dynamic alterations.

Excellent general discussions of such subjects as orthostatic hypotension, athlete's heart, idiopathic cardiac hypertrophy, beri-beri heart disease, pheochromocytoma, etc. are given. In the discussion of the cardiovascular system in uremia the author points out hemodynamic and EKG alterations like those produced by epinephrine. His own determinations of catecholamines appear to demonstrate elevated levels. Raab believes the "false positive" benzodioxane and Regitine tests in uremia have their basis in this demonstration.

On first perusal Raab's unorthodox ideas and unconventional presentation produce an unfavorable reaction. On closer analysis it becomes clear that his book has much that is

sound. Although perhaps not as important as Raab would lead one to believe, the factors he emphasizes are certainly of genuine significance and have for the most part been neglected hitherto. We need more unorthodox thinkers in this and most other areas of medicine.

A main usefulness of the book will be in its thought-provoking properties. A second important usefulness will derive from the bibliography of staggering proportions. With over 3700 individual references this part of the book occupies about one-fourth of the total page space. A more detailed compendium of the literature on the subjects covered by the book is hard to imagine. It will be all the more useful to American workers because of the substantial proportion of little familiar European references. Occasionally, when even most inconsequential statements are supported by a reference or two, this aspect of the book approaches the absurd.

The presentation is rather ponderous and may become irksome to the reader. One gets the feeling that the author could have presented his thesis more effectively in a much less lengthy monograph. All in all, the reviewer believes that those working in the cardiovascular field can profitably spend many hours pondering the ideas developed in this book; furthermore, they will find it a handy guide to unfamiliar work in this field.

VICTOR A. MCKUSICK

Medicine, Vols. I and II. Edited by HUGH G. GARLAND AND WILLIAM PHILLIPS. Foreword by F. A. E. Crew. 2146 pp., $25.00. St. Martin's Press, New York.

The following passage is quoted from F. A. E. Crew, who wrote the foreword of this new textbook.

"Medicine is involved in the maintenance or restoration of harmony within the individual, between the individual and the group or groups of which he is a member and between the individual and group and the components of their external physical worlds. The purpose, the aim, the social function of medicine are thus defined and the vastness of the territory of medicine revealed; it includes everything, literally everything, that in its action and effects upon man corrupts or tends to corrupt human perfection of body, mind and spirit."

This is indeed a large order for any single work to cover with proper emphasis on each of the topics discussed. The present work makes a very valiant attempt to fulfil the order, however, and in so doing departs from the purely scientific approach of the current American text-books to enter into full discussions of the environmental, social, genetic, and psychosomatic aspects of illness.

Since the work is composed of only two volumes and 2146 pages, it has been necessary for the editors to abbreviate the writings on some topics in order to cover the large field chosen for discussion. In most instances this has been done with good judgment, but the social aspect of disease has distinctly gotten more emphasis than it deserves in my opinion. For example, a chapter entitled "Effects of Severance of Relations during Illness" is given 9 pages, whereas disorders of the parathyroids and adrenals combined totals little over 7 pages, typhoid fever little over 5 pages, syphilis about 10 pages, etc. The worst feature of the work is the absence of any reference to important literature.

Some of the classifications used are unusual in this country also, for example the labeling of measles a respiratory infection rather than a virus disease, and the grouping of typhoid, paratyphoid, brucellosis, bacillary and amebic dysentery as "Ingestion Infections." Allowing for the difference in approach, however, the content of the text is excellent.

ROBERT E. MASON

Experimental Studies in Psychiatric Art. By E. CUNNINGHAM DAX. Foreword by Sir Steuart Wilson. 100 pp., $5.00. J. B. Lippincott Co., Philadelphia, Pa.

The author presents a summary of four years work in the use of painting and music in the treatment of chronic mental disorders. The approach of the book is descrpitive for the most part, making an effort to standardize techniques, in order that in the future, conclusions as to relative therapeutic efficacy could be made. There is an interesting brief description of the art work of a number of patients and the study of the situations producing the paintings.

This book should be of interest to anyone doing occupational therapy in psychiatric hospitals and to any psychiatrist or others in this field interested in more understanding of artistic communications. The book's most important contribution is the attempt to standardize techniques for study of therapeutic effectiveness.

ALLAN R. MCCLARY

Annual Review of Medicine, Vol. IV. WINDSOR C. CUTTING, Editor. Henry W. Newman, Associate Editor. 452 pp., $6.00. Annual Reviews, Stanford, California.

The extension of the Annual Review into the field of medicine seems to have been a success, for the fourth volume approaches each segment of its task with continued vigor. As one peruses these individual reviews one is impressed with the slow and piecemeal nature of advances in medicine. The tremendous difficulties of clinical evaluation make it hard to know whether ACTH is of real value in all sorts of burns, whether there are any clinical situations in which antibiotics are antagonistic, to know whether a new amoebicide is as good as some of the "tried and proven" remedies.

Such difficulties impose a special burden on the reviewer to be more critical and selective. Some of the good reviews in this edition are Infectious Diseases by Gillett, Diseases of the Cardiovascular System by Bing, and Nutrition by Cuthbertson.

FREDERIK B. BANG

BOOKS RECEIVED FOR REVIEW

Bakteriologische N¨hrböden. By LOTHAR HALLMANN. 252 pp., Ganzleinen DM 19.80 Georg Thieme Verlag, Stuttgart, Germany.

Die Wirbelsäulentuberkulose und Ihre Differentialdiagnose. By J. E. W. BROCHER. 272 pp., Ganzleinen DM 70.20. Georg Thieme Verlag, Stuttgart, Germany.

Introduction to Laboratory Chemistry, 4th edition. By L. EARLE ARNOW. Revised with the Assistance of Marie C. D'Andrea. 108 pp., $1.50. The C. V. Mosby Co., St. Louis, Missouri.

Introduction to Physiological and Pathological Chemistry, 4th edition. By L. EARLE ARNOW. Revised with the Assistance of Marie C. D'Andrea. 508 pp., $3.75. The C. V. Mosby Co., St. Louis, Missouri.

The Major Metabolic Fuels. (No. 5 of a series of symposia sponsored by the Biology Dept. Brookhaven National Laboratory, Upton, N. Y.). 234 pp., $1.35. Associated Universities under contract with the United States Atomic Energy Commission.

Plant Proteins in Child Feeding. Medical Research Council Special Report Series No. 279. By R. F. A. DEAN. 163 pp., $2.25. Her Majesty's Stationery Office, London, England.

Problems in the Anatomy of the Pelvis. An Atlas. By EDUARD UHLENHUTH. 206 pp., $10.00. J. B. Lippincott Co., Philadelphia, Pa.

Psychotic and Neurotic Illnesses in Twins. Medical Research Council Special Report Series No. 278. By ELIOT SLATER. 385 pp., $4.75. Her Majesty's Stationery Office, London, England.

Renal Function. Transactions of the Fourth Conference Oct. 22, 23 and 24, 1952. Edited by STANLEY E. BRADLEY. 189 pp., $3.50. The Josiah Macy, Jr. Foundation, New York.

Report of the Medical Research Council for the Year 1951–1952. Presented by the Lord President of the Council to Parliament by Command of Her Majesty, June 1953. 241 pp., $1.50. Her Majesty's Stationery Office, London, England.

Some Conjugated Proteins. Edited by WILLIAM H. COLE. 73 pp., $1.75. Rutgers University Press, New Brunswick, N. J.

Zur Genese des Diabetes mellitus und des Bronchalasthmas. By M. BAUER. 113 pp., Kartoniert DM 9.60. Georg Thieme Verlag, Stuttgart, Germany.

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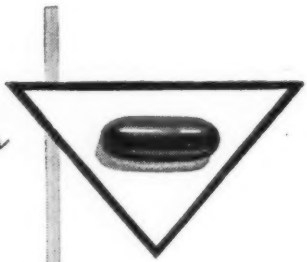

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